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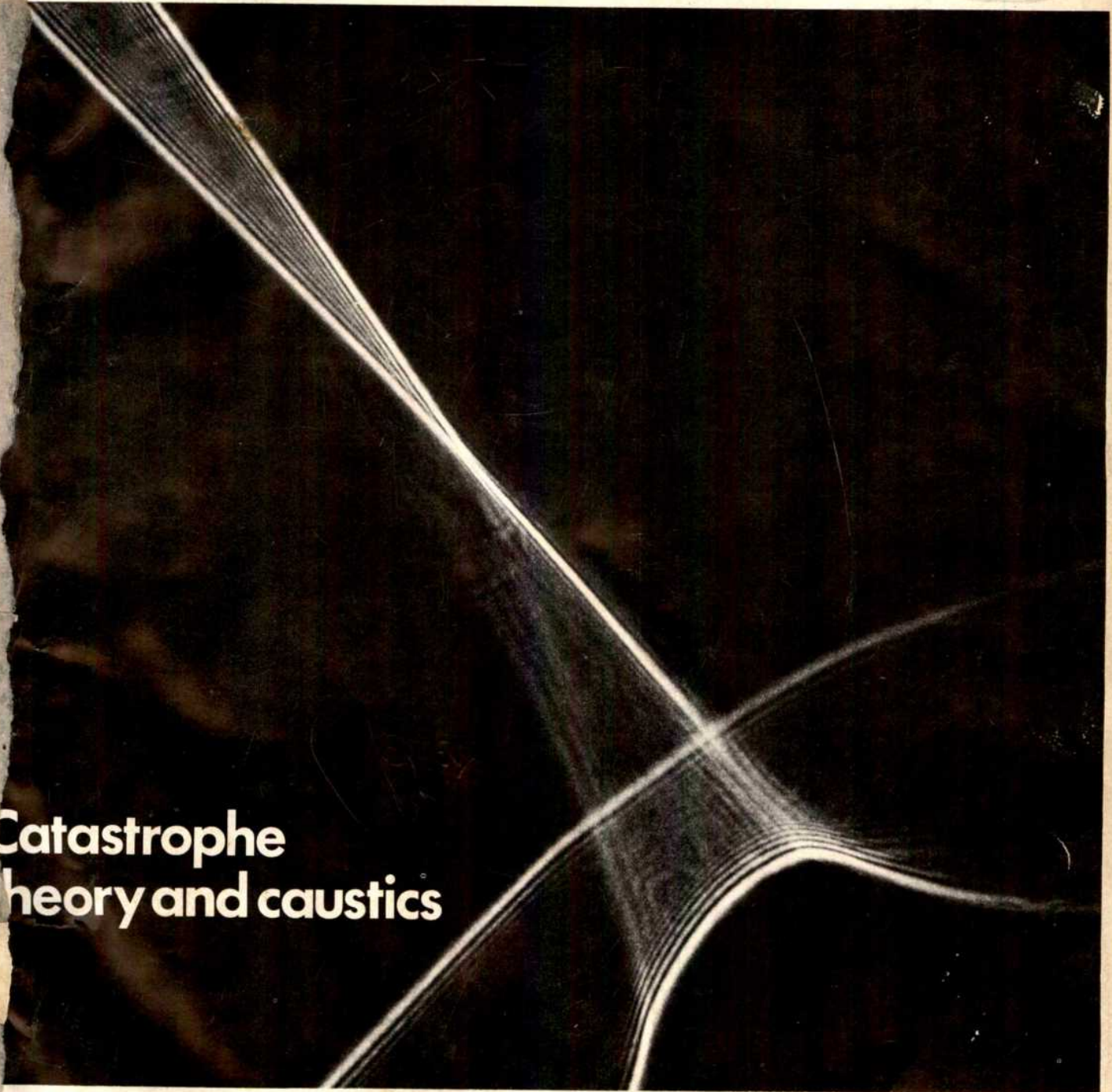
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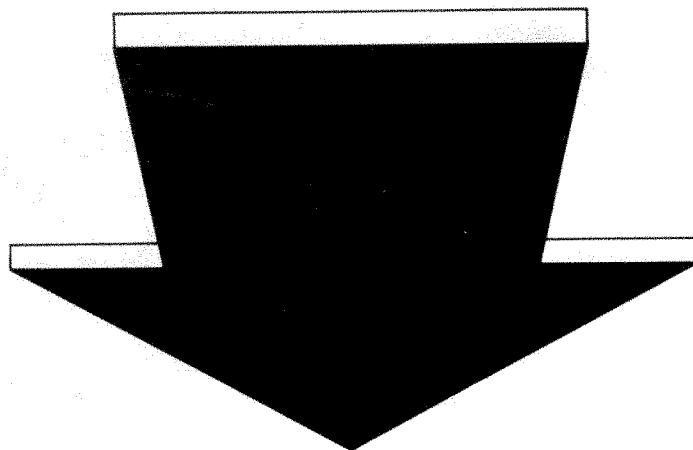
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**Catastrophe
theory and caustics**

**Accelerators
or the future**

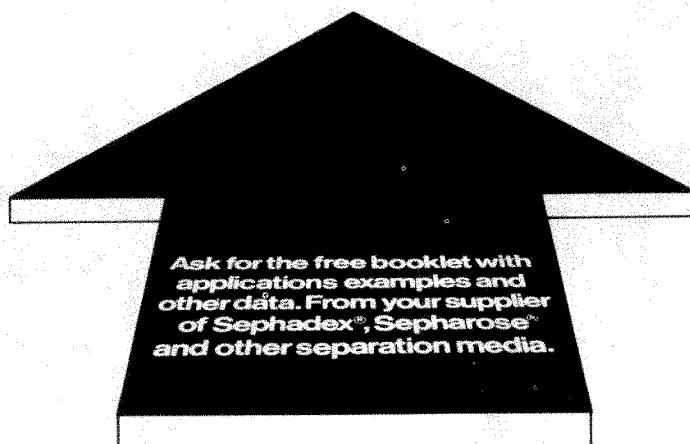


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A Darrieus vertical axis windmill as
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John Donat

Martin Ryle writes on the economics
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Bedouins planting eucalyptus trees
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[Werner Braun, Camera Press]
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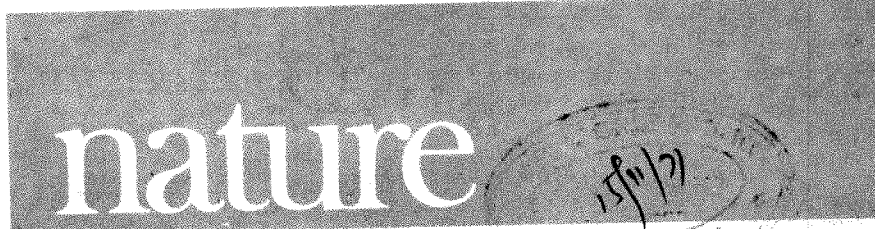
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Volcano in the Tolbachik area
of Kamchatka.

On page 420 Drs Livshits and
Bolkhovitinov describe observations
they have made of shock waves in
the eruption column
[L. D. Livshits]

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Cover picture

Iceberg at Cape Royds.
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The mammary duct system of mouse
breast (photographically reversed).
Chemically-induced breast cancers,
most of which arise in this system,
can be inhibited by a natural form of
vitamin A (see p.620).

(Ritva Evarts and Carolyn Brown.)

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The Rutherford Laboratory Central
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Golden *Tubastrea* coral polyps
opening at night.

(Photo: Jane Burton/Bruce Coleman Ltd.)

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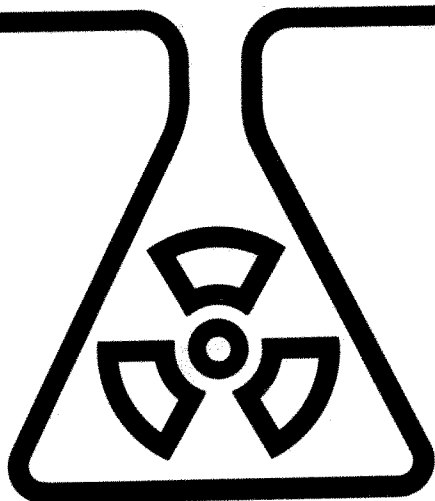
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'Nation shall speak peace unto nation'... at -30 dB

How should television develop in the next ten or twenty years? On the technical side, it goes without saying that most people would like to see every economically visible development explored: cable television, satellite broadcasts, use of the receiver for access to news, data and computers, new types of television screen and so on. But on the question of programming there would be a variety of answers, as the recent Annan Committee has found in trying to formulate recommendations on Britain's fourth channel. Some want more of the same, some want education programmes, some argue that more inevitably means more. There is little doubt, however, that most (and this not likely to be just a British view) would like more choice if there were some guarantee that this did not mean rationing of resources. One fascinating way in which this could have been achieved by enabling viewers to tune into other countries' programmes is likely to be suppressed before the general public is even aware of the possibilities.

At present, of course, television reception in the hundreds of MHz range is restricted by line of sight. The technology for transmission in the 11.7 to 12.5 GHz band from geostationary satellites is advancing rapidly, however, and there is talk in some countries of satellite broadcasting within fifteen to twenty years. At the same time, pressure is growing, but not in every country, for the allocation of frequencies in the same band to terrestrial services. An internationally agreed decision in 1971 gave priority to satellite broadcasting in choice of channels and this has meant a global gathering at Geneva earlier this year to allocate channels long before the precise needs can be foreseen. And since land-based services will develop well ahead of satellite broadcasting, the latter, now allotted its channels, will find itself in a straitjacket. The 20 MHz bands can certainly be used for radio as well as television, but a large screen digital receiver, which some describe as the television of the future, might well need greater bandwidth. It is not the technological straitjacket, however, which is most disturbing—it is the political constraint that programmes are not to cross frontiers.

As envisaged at present, each broadcasting nation would have its own satellite which would be at liberty to transmit on up to five channels, at 80 MHz spacings. The beams from satellite antennas would have a minimum width of 0.6° (which would more than cover Ireland) and could be elliptical (a beam $1.8^\circ \times 0.7^\circ$ would cover the UK). Every country in Europe, including Luxembourg, the Vatican, Andorra, Monaco, Liechtenstein and San Marino, has been solemnly awarded its five channels; in the case of the

smaller countries (in which there is arguably not quite enough talent to provide five simultaneous programmes and equally arguably adequate line-of-sight to surface transmitters), if they chose to launch a satellite the smallest beam possible would provide neighbouring countries with ample, if accidental exposure. On the other hand countries that wish to use one or more channels for deliberate broadcasting beyond their frontiers with broader-than-necessary beams have to obtain prior agreement from countries they wish to serve. The Scandinavian countries have been able to come to such an agreement, and the Vatican, Tunisia and Saudi Arabia (to serve Islamic countries) have also been allocated 'superbeams'. But other requests were turned down. Ireland wished to cover the UK for the sake of the large Irish population, but the UK claimed they had not had enough notice. And none of the major countries at the meeting seem to have made any public noises about broadcasting general television programmes over a wide area; indeed it is fairly widely known that the Eastern Europeans and France would refuse permission for such an invasion of their domestic life.

The objections are fairly obvious.

- Television could deliver some pretty slick propaganda; indeed it could, but the public on the whole switches propaganda off, and in any case an international channel might sensibly be compiled only of material which is also being shown domestically.
- There are technical problems of channel assignment and of having to direct an antenna at more than one satellite; hardly crippling problems in a technological age.
- The whole thing would only appeal to a tiny minority; well you wouldn't have to buy a multidirectional aerial if you didn't want to, and who is sure that foreign sport, music, entertainment and documentaries would not catch on? Twenty years ago Indian food and Spanish holidays were only for a tiny minority.
- We can already see foreign programmes; yes what 'the authorities' decide to accept on our behalf.

Too many conflicts in the past and too many prejudices in the present are based on the caricatures that most nations have of fellow-nations—caricatures largely fed by editorial control of the media. Here would have been a marvellous opportunity to ensure that at least the next generation could learn a lot more about the world as a whole—and maybe be stimulated to learn more foreign languages. But it has been thrown away. The next round of frequency discussions is probably ten or fifteen years in the future and will be too late. □



Europe's pollution politics

Chris Sherwell reviews some of the problems in producing an EEC environment policy

THE achievements of international organisations are, in large measure, a political trade-off between what is theoretically desirable and what is practically possible. Unsurprisingly, the balance is usually in favour of the latter. In spite of, and perhaps because of their unique but complicating commitment to political and economic union, the nine member countries of the European Community (EEC) display the attribute more than most organisations. Nowhere is this more amply demonstrated than in environmental policy.

The EEC's very involvement in the field is under challenge. When the European Commission last year produced its Second Action Programme on the Environment, covering the years 1977 to 1981, one of the most powerful of the environmental lobbies, the European Environmental Bureau (EEB), representing some 38 organisations throughout the Nine, weighed in with a powerful critique. There was a contradiction, it contended, between the main goal of the EEC, which was continuous and balanced economic expansion, and the basic principle of ecology, which was a dynamic equilibrium.

In fact the EEB displays all the signs of being pleased with the EEC involvement in environmental matters. The second action programme, after all, reads much like an environmentalist's manifesto, so breathtaking is its broad sweep. The EEB's reservations concern the precise character of that involvement. It criticises, perhaps with some justification given the programme's breadth, the curious lack of attention given to transport, population growth, regional policy and national programmes. This sort of gap, it is intimated, begs its own questions and reinforces the contradictions.

Questions are indeed necessary.

Environmental issues find under an office in the Commission with split responsibilities—the Environment and Consumer Protection Service. Its staff numbers around 100. Environmental groups would like to see greater status attached to the department, perhaps making it a Directorate General. More important and perhaps contradictorily, they would like to see its work better integrated in the activities of the Commission and Community as a whole.

The argument is that the EEC's involvement in the environment will be ineffective if it is limited to drawing up a programme regardless of what the various departments of the Commission are doing, as now seems to happen. The aim ought to be to promote the concept of environmental quality in all the departments, so that the work done by an environment department is not emasculated by the activities of other departments. An environment protection department, in short, ought to participate in the policy making of other departments.

In spite of this very real structural problem facing the Commission, there are people who will praise its work; they recognise the constraints, both manpower and financial, that it faces in trying to coordinate the activities of nine countries across a broad range of environmental and other matters. But its achievements in such adversity are in turn the spring of other equally penetrating criticisms of the environment policy it administers. These emphasise the need for selectivity as a paramount requirement for success. The argument is that the lack of focus, the effort to do "too much too soon" and "run before being able to walk", is a fundamental cause for disillusion.

A glance at the second action programme is certainly enough to convince

even the most ambitious lobbyist that the chances of it being legislated in fifteen years, let alone five, are at best remote. This means that the sense of priority can become blurred, and emphasises the need for greater clarity about what the Community can do and what its role can be. After all, it is not just the Commission whose institutions may need modifying to encourage a well-rounded environment policy; it is also the Council of Ministers, before which the issues may come somewhat haphazardly and which finds the greatest difficulty in dealing with them systematically and without awkward and embarrassing haggling. The point is, not all environmental matters demand a specifically European approach.

Techniques of control

If the question of the degree to which the Community should become at all involved in environment policy seems important, it does not exhaust the list of fundamental questions on the subject which it currently faces. The very techniques of control are another issue, and form the basis for the longest running political controversy of all in this area.

Even boiled down, the essentials of the European debate are far from straightforward. The environment is recognised as having some capacity to receive pollutants. Where risks are apparent but complete prohibition is unnecessary, controls are needed. Two forms come under scrutiny, known in Europe as 'quality standards' and 'quality objectives'. Put most simply, quality objectives are pollution levels which the nine member states each undertake to do their best to attain, and quality standards are levels which the nine states each become obliged to achieve. The debate is about how precisely to use them.

Going by the political argument as it has developed thus far, the difference looks more than merely legalistic. For, having painstakingly arrived at a view of what the standards or objectives should be, the Commission is able to take steps to assist in their attainment by establishing what in its jargon are called 'norms'. This, it seems, is where the trouble starts, because it has meant the introduction of the notion of emission standards, to which Britain has strongly objected and other states and the Commission have given support.

The characteristic of emission standards causing Britain to dig its feet in is not that they are to be used, but that they are to be used uniformly. Britain has supported quality objectives on the grounds that they can take account of the varying nature of the receiving environment in a way that standards, because of their intrinsic

uniformity in the Community, cannot. To Britain, quality objectives imply emission standards but not uniform emission standards. Once it is agreed what quality a particular stretch of water, say, ought to have (which in turn depends on the use to which it is put), a quality objective must be set. To reach this, emission standards will need to be set for particular polluters on that stretch of water, but they will be peculiar to that stretch of water being used for that purpose.

Not so, say the hard-line Europeans. The imperfections of quality control mean that the only reliable path to reductions in pollution is to declare emission standards for particular classes or sections of industry and apply them uniformly throughout Europe; such standards would have to be achieved by a certain time. The British version of an emission standard, they say, is effectively a licence to pollute.

The arguments have in fact found their most coherent expression in the debate over aquatic pollution, itself just one aspect of pollution generally and a mere fragment of the environment question. Pointing to its shorter rivers, larger coastline and smaller and lower concentration of factories, Britain has argued that it can have pure water without adopting on a uniform basis the higher emission standards that would be demanded on mainland Europe. Other countries, with an eye to the Community's competition policy, have said this would give Britain an unfair competitive advantage in international trade and jeopardise a common policy both in competition and environment matters. Britain has contended that its advantage derives from possession of a natural resource and that a uniform policy would be wasteful, only harmonising costs rather than encouraging fair competition.

This dispute has occupied a considerable amount of important time. In part this is no doubt because of misunderstandings. For example, at a seminar organised in London recently on EEC environment policy, one Commission official cited the hypothetical example of two states in exactly similar circumstances trying to achieve the same air quality, in one instance by tackling only a few big companies and in the other by tackling many small companies; this, he said, would amount to an unacceptable distortion of competition. A surprised British official found that the example fitted with his own country's view of having the same environmental responsibilities in the same circumstances.

Much of the debate as it has involved aquatic pollution has necessarily involved the so-called 'black list' and 'grey list' of dangerous substances,

classified on the basis of toxicity, persistence and accumulation. Put most simply, the idea is to isolate black list substances from the environment to the point where there can be no doubt that they will harm human health, but some countries have said that black list substances should not be dumped or discharged at all. Others doubt the need for this, given the uncertainty about the substances themselves and about the absorptive capacities of the environment.

Although the fundamentals of the argument remain essentially unresolved, a breakthrough did come at a meeting of the Council of Ministers in December 1975 when a compromise solution was reached on black list substances. This embraced a dual approach allowing either emission standards on a uniform Community basis, or quality objectives which catered for different circumstances. Formally the compromise retained the notion of uniformity and allowed temporary exemptions for which Community but not uniform standards could be used: the exceptions would arise when a member state could prove that quality tests were being maintained throughout the particular area concerned, and would be re-examined every few years; the whole system would become operational within about three years by which time priorities would be decided and actual objectives and standards set. Even then it might take five or ten years to achieve, and in light of this Britain views the application of the quality objectives as unlikely to lag much behind the implementation of emission standards.

For the grey list it was agreed that individual member states would prepare programmes which included quality objectives, from which they would derive emission standards of their own. The Commission would ensure that comparisons between countries were made to ensure some uniformity of approach, but the underlying approach became one involving quality objectives, and there is no mention of emission standards on a uniform Community basis. The recent seminar in London may anticipate an even broader acceptance of the British view.

Fears persist that the black list will in effect become a grey list. However, the detail of the breakthrough, though important, was less important than its immediate consequences. Progress could be made in April 1976 towards a Rhine convention covering pollution by salts, chemical pollution, thermal pollution and pollution by radioactivity. Agreement on limits of chemical pollution was soon reached, and a month later ministers met again to resolve

differences over salt pollution. Progress was also possible on the matter of Mediterranean pollution. In Barcelona in February 1976 16 countries finalised details of a framework convention and two protocols covering the discharge of petroleum waste and the tipping by ships and aircraft of black list and grey list pollutants into the Mediterranean.

Achievements not matched

These 'international' achievements involving Community members have not since been matched within the Community itself. At the most recent Council of Ministers meeting last December, for example, four draft directives from the Commission awaited approval: on the biological surveillance of the population for lead in the blood; on the quality of water intended for human consumption; on the waste from the titanium dioxide industry; and the reduction of water pollution by paper pulp plants. The Council adopted only the first of these.

At the end of last year, in fact, there was still around a dozen sets of proposals for directives sitting on the Council's desk awaiting action. They included proposals relating to the lead content of petrol (submitted 7 December 1973), the dumping of wastes at sea (submitted 12 January 1976), health protection standards for sulphur dioxide and suspended particulate matter in urban atmospheres (submitted 25 February 1976) and toxic and dangerous wastes (submitted 28 July 1976). Proposals for decisions awaiting adoption included one on the subject of organic micro-pollutants in water and another on exchanging information on the quality of surface fresh water in the Community.

This last item is expected to appear on the agenda, along with the other undecided items from the December meeting, when the Council of Ministers meets in June for the one and only time during Britain's present chairmanship. Outstanding items will probably also be included, and perhaps some new ones like the conservation of birds, though the chances of them being considered remain as always subject to the will of the member states on the higher



Keystone

priority items.

In its programme for 1977, published in February, the Commission says it will concentrate on four basic 'priority themes': measures to combat water pollution; evaluation of measures to prevent the deterioration of the environment; an anti-waste campaign based on a policy to encourage materials recycling; and implementation of the international conventions on the Rhine and the Mediterranean. It says it will therefore send to the Council proposals for directives on, amongst other things, protection of underground water, the quality of water for agricultural use, notification of industrial

activity involving dangerous substances (a lesson of Seveso), discharge of various particularly harmful pollutants, and on anti-noise measures.

The Commission remains optimistic. Its work goes ahead under the auspices of the second action programme, which the Council meeting of last December unanimously approved, adding the undertaking that it would act on concrete proposals from the Commission within nine months of them being forwarded. It may prove to be a tall order, given the fundamental character of some of the issues still outstanding concerning the Community's environment problem. But the

Community and its policies are nothing if not resilient. And the large number of directives which have already gone through suggest that the whole effort in the environmental field may have acquired a momentum.

That should allow some attack in the primary field of preventing and eliminating pollution and nuisances, where most attention has been concentrated. But managing the use of natural resources properly, controlling the ecological balance and economic growth, and generally protecting the biosphere—also aims of the EEC's second action programme—will plainly take a little longer. □

Spreading the word

David Spurgeon, recently in Asia, examines the growth of science writing there

THE business of writing about science for the mass media must have its ups and downs like any other business. Although it is difficult to judge without the evidence of a full-scale survey, it seems the trend in North America in recent years has been predominantly down. Editors no longer show the enthusiasm for science subjects that they did during the early post-Sputnik days and discussions have appeared in science writers' newsletters about the parlous state of their craft.

Some link the decline in interest to the scepticism—and in some measure, disillusionment—about the promise of science that followed the excesses of publicity associated with the US manned space programme, the development of nuclear power and 'wonder drugs' and other achievements. It seems a reasonable thesis; the earlier 'gee-whizz' phase of science writing was followed not only by a much more critical and judgmental phase, but also by the birth of various anti-science movements and by 'consumerism' and 'environmentalism'.

An interesting contrast is found in the Third World, particularly in Asia, where (again to some extent subjectively) interest in science journalism seems definitely on the upswing. Mack Laing, a Canadian science writer and professor of journalism, writing in *Depthnews Science Service*, a weekly newsfeature service of the Press Foundation of Asia (PFA), put it this way in a recent article from Manila:

There is a burst of activity in Asia these days toward achieving a greater public understanding of science. Some—perhaps even most—Asian nations are just waking up to the need for more public appreciation of how science and technology can help national development. Other countries

are going full blast on the same. Leaders in these countries argue that popularising science encourages people to understand and cooperate with government actions on large-scale problems such as conservation, pollution, irrigation and flooding by man-made reservoirs, sanitation and infectious diseases. They argue that interesting the youth of a country in science increases the country's science manpower and speeds national development. One way of bringing the word about science and technology to the people is through the media of mass communications.

The *Depthnews Science Service* and Mr Laing's activity in it are themselves interesting results of his newly-awakened interest. PFA, a non-profit agency founded by Asia journalists some ten years ago to improve the standards of the region's media, asked Canada's International Development Research Centre (IDRC) for funds to set up a science feature service within *Depthnews*. Its aim would be to set the pace for the Asian press in science coverage, which in its opinion (and that of other Asians) had been deficient until then. PFA also asked for an experienced science journalist from the West to set it up and train Asians in

the techniques of science journalism.

While it is too early to judge the commercial viability of the science feature service, the initial response of the Asian media has been surprisingly good. Mr Laing has found himself in the ironic position of having to admit that the interest shown by the media in Asia for his service's articles is much greater than he could imagine Canadian editors showing for a comparable service at home.

Among his chief sources of news in the Philippines are weekly news conferences set up specifically for science writers by the University of the Philippines and the National Science Development Board. The conferences were arranged to acquaint science writers with researchers whose work is sponsored jointly by these two organisations. It seems to be succeeding. At a recent conference, about ten media representatives gathered in a small room to hear a pharmacologist talk about work she and her colleagues had been doing in identifying plants with insecticidal qualities that could serve as replacements or alternatives for chemical pesticides. (The other subject, oddly enough, was music, but apparently this was atypical, almost all subjects being scientific).

To encourage attendance, the university has adopted the practice of paying the journalists to attend the con-



Sri Lanka seminar on popularising science, fiction writer Arthur Clarke speaking

ferences, which are held on Saturday mornings, and of giving them coffee and lunch. The sum is small (the equivalent of about \$9) and although the practice would obviously be unacceptable to the Western press, it may help (and perhaps be necessary) in the Philippines. Dr Joventino Soriano, the coordinator, first introduced the news conferences by holding a four-day science writers' seminar at a mountain-top retreat some miles from Manila. A travelling seminar was also planned in April, when media writers and researchers were to visit villages in three provinces north of Manila to see how science and technology can aid rural areas.

Similar seminars have been held by other organisations. In 1974, the IDRC held one near Manila for science writers. The response was strong from the media in eight regional countries, some of which already had science writers (and one, Korea, highly developed science coverage). In other countries (for example Indonesia) the interest was there but the media structure was not. In 1975, another seminar was held in India, with a similarly strong response from that country and others bordering it. A third seminar planned for June in Nairobi, will be for the East African media.

In March, a month-long Asian Training Course for Leaders in the Promotion of Public Understanding in Science, sponsored by UNESCO and the Science Foundation of the Philippines, was concluded in Manila. And in February, as a result of the previously mentioned India seminar, a meeting was held in Colombo, Sri Lanka, on 'Science writing for the people'.

In Sri Lanka, where science writing is largely ignored in English-language newspapers, possibly partly because newsprint shortages make for thin newspapers, the Ministry of Education is reported to be planning on providing scholarships for science writers to obtain university degrees.

At the recent seminar, Professor P. P. G. L. Siriwardene, the Vice-Chancellor of the University of Sri Lanka, said that science must be written in the vernacular to get to the people. But he added that popularisation of science has been "hamstrung by a lack of funds". In the developing countries, he said, science popularisation should be "an important national venture". The question of language is an important one, as delegates to the IDRC's first seminar noted. In many Asian and African languages there are no words for some scientific

concepts. However, in Sri Lanka, as in India, some science does appear in the vernacular-language press.

The Philippines is one of the most enthusiastic promoters of science popularisation among Asian countries. In addition to the emphasis on science news (which is perhaps attractive to editors because of government control of the country's press and the dearth of genuine news stories) there are hundreds of science clubs, which are joined in a federation and hold Youth Science Camps. Science campers visit rural areas to explain science and scientific concepts to farmers and remote villagers, and to replace superstition and myth by rational explanations of natural events. A science quiz, an annual high school contest, has attracted 8,530 regional participants and 292 national participants since 1969.

In a speech prepared for the course on public understanding held in Manila last March, President Marcos said:

We are belatedly finding out that the harnessing of science and technology is not a mere matter of learning formulae, but a process of cultural transformation itself. It is in this light that I perceive the importance of promoting wide public understanding of science, technology and the environment. □

RED SEA

Sifting the sediment

The staff of La Recherche reports on the prospect of mining sediment in the Red Sea

A CONTRACT was signed on 25 March, in Paris between the Red Sea Commission, represented by its secretary general, Dr Zaki Mustafa, and the Argas Company, represented by its president, Jamil Jawa. The contract, involving FF30 million (£1=FF8.54) is one of a series of agreements on the exploitation of the metalliferous sediment in the Red Sea, which contains 5.8% zinc, 0.90% copper and 110 g of silver for every ton of dry material as well as iron and manganese.

The first agreement between Saudi Arabia and the Sudan, signed in May 1975, was to cooperate in exploiting the mineral resources at the bottom of the Red Sea in the so called 'Common Zone', which lies between the two countries. The resulting Red Sea Commission, which has its headquarters at Jeddah, was founded to promote and encourage mining research and any means of exploiting the minerals in the zone. The Commission then called in the Argas Company (The Arabian Geophysics and Surveying Company, a joint

venture between the Saudi company Petromine and the French Compagnie Générale de Géophysique).

Two preliminary contracts in March and July 1976 (for FF 15 million and FF8 million respectively) covered geochemical, seismic and aeromagnetic studies. The work to be carried out under the third contract, the one recently signed, will analyse the environment including swell, currents, thermosalinity and biology to ascertain the effect of exploitation on the environment and to determine the conditions necessary for lowering a vertical tube for pumping mud to 2,200 m.

The metal extracted from the sediment would be treated on the spot, and mineral salts and tons of residue extracted from the metal ore would be thrown back into the sea; but what would become of this residue, and what effect it would have on the flora and fauna before settling back on the sea bed remain unanswered questions. A German company, Preussag, has meanwhile been developing the extraction process. A pilot preconcentration plant could be working by 1982, and it will not be known until then whether the project is feasible. Preussag is also under contract from the Red Sea Com-

mission, which is providing \$25 million for six years. The Commission has engaged the services of a French public body in charge of geological and mining research, the BRGM, to coordinate activities and act as technical adviser.

If the project succeeds, it will be the first time that marine sediment has been put to use: other companies engaged in working the ocean bottom are more interested in nodules. The sediment contains important components, however, especially copper, and similar deposits could be found in other parts of the world. Geological studies provide an explanation for the formation of mineral rich sediments: in the Red Sea, where rock outputs cut across the rift, there is a zone of intense volcanic and thermal activity, and the rocks give off large quantities of metallic salt into the water. In the presence of the hot brine frequently found in these regions the metallic salts are eventually precipitated. Thus a sedimentary bed is formed containing enough metal for it to be thought worth while mining out. The first mining will be in the Atlantis II fault, where it is estimated that there would be 850 million tons of sediment, that is 7×10^6 tons of copper. But at least 18 faults are known in the hot brine regions of the Red Sea; some probably do not contain sediment, but others could be extremely profitable. □

USA

Going public on rights

Colin Norman reports from Washington on an appeal in support of named scientists who are victims of political repression

In a significant departure from its usual style of quiet diplomacy, the National Academy of Sciences last week called a press conference to urge public support for eight scientists in three countries who are "victims of grave official harassment for political reasons". The action, taken by a newly-established Committee on Human Rights, signals that the Academy is prepared to adopt a more aggressive role in defending scientists against political repression than it has in the past.

The eight scientists involved in the appeal are five Argentinian physicists who disappeared in 1976, a Uruguayan mathematician arrested in October 1975, who has been charged with subversive activities and allegedly tortured, and two Soviet scientists, one of whom is serving a prison term and the other is under arrest awaiting trial. According to Robert W. Kates, the chairman of the Academy's Committee on Human Rights, these eight scientists are the first of "a large group" which the Academy hopes eventually to take public action on. "They are", said Kates, "at best symbolic of the many scientists who are subject to repression".

The Committee on Human rights was established a year ago, following complaints from some Academy members and from the Federation of American Scientists (FAS) that the Academy had been too timid in its attempts to defend scientists against acts of political repression. Though it made one highly publicised statement in support of Andrei Sakharov in 1973, the Academy has worked mostly behind the scenes, making private representations to officials of foreign governments on behalf of harassed scientists. Early last year, there was a move, sparked by complaints by the FAS Director, Jeremy J. Stone, to urge the Academy to back up its private negotiations with occasional public statements.

The Academy responded by establishing the Committee on Human Rights and it also adopted a set of guidelines which suggested that the Academy would speak out publicly when the occasion so demands. It seems from last week's press conference that such public appeals may become more common. Kates said that the committee will concentrate on in-

dividual cases rather than making broad statements of principle, and he indicated that several more cases will be added to the list as soon as further evidence is collected. "This is just the beginning", he said.

Asked whether the committee would be prepared to urge the Academy to take further steps if the appeal has no effect, Kates said that the committee would proceed one step at a time. The ultimate sanction available to the Academy would be to back out of an exchange agreement with a country which persists in acts of political repression against scientists. In any case, there seems to be a strong body of opinion within the Academy supporting the committee's moves so far. Kates said last week that a letter sent to Academy members asking for their support in writing to foreign officials to protest acts of political repression produced 258 positive responses.

The specific action taken last week was to ask scientists to write to officials in Argentina, Uruguay and the Soviet Union in support of the eight scientists.

In Argentina, the five physicists are Federico Alvarez Rojas, Gabriela Carabelli, Juan Carlos Gallardo, Antonio Misetich, and Eduardo Pasquini. All disappeared during 1976, presumably arrested, but the government claims to have no knowledge of them. The Academy is asking that they be permitted to see their families and that scientists be allowed to visit them to determine their state of health. In addition, the Academy is calling for the physicists either to be charged or released and that observers be permitted at any trial. In fact, however, members of the Academy's committee said that there is no guarantee that the five are even alive.

The Uruguayan mathematician, José Luis Massera, was arrested in October 1975, held incommunicado for a year and is now being tried in camera on charges of "subversive association". According to Lipman Bers, a member of the Academy's human rights committee, Massera, who is 62, is "the most distinguished mathematician in Uruguay" and he has a worldwide reputation. An avowed Communist, Massera has been "subject to severe torture", Bers said. In this case, the Academy is again asking for permission for Massera's family members and representatives of the world community of scientists to visit the mathematician, for observers to be permitted at his trial, and for him to be given access to books and other scientific material.

The two Soviet scientists are Sergei Kovalev, a biologist who is now serving seven years' hard labour and three years' exile for alleged activities against the Soviet state, and Yuriy Orlov, a physicist who was arrested in February 1977 and is awaiting trial on as yet unspecified charges. In Kovalev's case, the Academy is concerned about his ill health, and is appealing for commutation of his sentence to the time already served. The Academy is also requesting scientists to appeal directly to the Soviet authorities on Kovalev's behalf and to provide Kovalev himself "with every feasible manner of moral and professional support". As for Orlov, he has been a leading participant in the Soviet human rights movement associated with Sakharov, and was arrested as part of the recent crackdown on the dissident movement. The Academy is inquiring about his legal status and place of detention through a letter sent last week to the Soviet Ambassador in Washington, Anatoliy Dobrynin, and the human rights committee will "take appropriate action".

The Academy's new, more aggressive policy puts it in marked contrast to the Royal Society. In a speech to Royal Society Fellows last November, Lord Todd, the Society's President, expressed concern about the widespread political repression of scientists in many countries and about more subtle infringements of scientific freedom, but he said that the Royal Society should steer clear of making public pronouncements on the matter. Instead, Todd said that Royal Society officials would continue to make private representations on behalf of scientists whose human rights are being infringed.

Further details of the individual cases raised by the Academy last week, and addresses of government officials to whom correspondence should be addressed, can be obtained from the Committee on Human Rights, National Academy of Sciences, 2101 Constitution Avenue NW, Washington DC 20428. □



Sergei Kovalev

USSR

● The raising of quality standards is a major watchword of the current Five-Year Plan and a slogan for the forthcoming celebrations of the Diamond Jubilee of the Soviet Union. In the traditional panegyric of Soviet achievements given on Lenin's birthday (22 April), Mikhail Zimyanin, Secretary of the Central Committee of the Communist Party of the Soviet Union, noted among other achievements an improvement in the quality and efficiency of research and the coordinated study and solution of problems of scientific and technological progress. A major re-equipping of industry is under way, he said, and in spite of "difficult weather conditions" the last year has been marked by "new outstanding achievements of the Leninist agricultural policy of the Party".

Nevertheless, in at least some of the Union Republics, there is less emphasis on the implementation of the plans. In Lithuania, according to the First Secretary of the Central Committee of the Lithuanian Communist Party, there is a tendency for certain ministries and departments to draw up "easy" plans and adjust the targets downwards. The First Secretary of the Central Committee of the Byelorussian Communist Party, speaking at the recent Plenum, told a somewhat similar story. Some bodies including research institutes and the state statistical organs, he alleged, keep up a front of activity, busying themselves with data-gathering and the preparation of summaries rather than with the "scientifically substantiated recommendations" which the economy requires; even when recommendations were made, the proper experimental basis might be lacking.

He cited in particular a new system of product quality control for dairy farms which was recently worked out by the Byelorussian Scientific Research Institute of Agricultural Economics and Organisation and which has been widely publicised. However, the whole experimental basis of the scheme apparently rests on "a brief experiment on only one farm", reflecting a lack of practical trials reminiscent of the extremely scanty foundation for Lysenko's "vernalisation" scheme. Although not criticising in so many words the system of targets which is the basis of Soviet planning, the Byelorussian first secretary deplored the tendency to set targets without proper evaluation of the "qualitative parameters of economic development".

● The Dubna Joint Institute for

Nuclear Research has completed a cycle of experiments on the synthesis of element 107 by bombarding ^{209}Bi with nuclei of ^{54}Cr . The first experiments, in 1975, gave strong evidence of the formation of the element with a half-life of 2 milliseconds. The Dubna team, under Academician Georgii Flerov, has already syn-



thesised elements 103–106. In announcing the successful conclusion of the current series of tests, Academician Flerov gave a strong hint that they now would be aiming at elements of even higher atomic numbers—a new unit is being built, he said, which will increase the efficiency of nuclear interactions by a factor of several hundred.

● One of the major features of recent Soviet work in the Antarctic has been the drilling of deep cores both through the ice-shelf and on the continent proper. A special electrothermal core drill is used, with a maximum penetration of 6 metres an hour with, when necessary, a special system for keeping the core sterile. All apparatus is easily transportable by sledge or helicopter, hence cores may be taken if required at a considerable distance from the base.

The ice-shelf project was based on the Novolazarevskaya station (Queen Maud Land). The purpose of the experiment was not simply to penetrate the ice but also to sample the sea-water and sea-bed below. The first such shafts gave the depth of water beneath the ice as 40 m, its salinity approximately that of the open ocean and its temperature -1.5°C to -1.9°C . The sea-bed specimens indicated that the composition of diatoms deposited had not

changed during the past 10,000 years.

Inland drilling operations were based on the Vostok station at the 'Pole of Cold', where the icecap is some 3,500 m thick. A 980 m core from this area has been analysed by the ^{18}O method. According to Professor Evgenii Korotkevich, who is one of the supervisors of this project, the core indicates a palaeo-climatic pattern with a mean annual temperature at 15,000 B.P. some 5° below the present figure, indicating a significant drop in the world temperature. This, he says, was followed by "rapid reconstruction" of the climate, thought to be associated with cosmic phenomena, with the present climatic conditions becoming established *circa* 11,000 B.P., since when the climate has remained virtually unchanged.

The Vostok station is also the base for taking sterile cores for experiments on the 'reanimation' of microorganisms. The cores from the twentieth expedition (Summer 1975–76) penetrated to 207 m, the most ancient organisms reanimated being from the 197 m level ($\sim 8,500$ B.P.). From the specimens of the twenty-second expedition (Summer 1976–77), it is hoped to establish the limit of survival of microorganisms in the anabiotic state, a topic, it is said, in which the Laboratory of Space Microbiology of the Microbiological Institute of the Soviet Academy of Sciences is showing considerable interest.

● The case of Anatolii Shcharanskii, at present under arrest pending investigation, is a peculiarly ironic example of the 'secrecy' issue which faces scientists wishing to emigrate to Israel. Shcharanskii, who is prominent both in Jewish refusenik circles and in the larger human rights movement (he is a member of the banned Helsinki monitoring group), studied at the Moscow Institute of Physics, specialising in computer science. He has not, however, ever worked at his profession.

Because the research post he was offered at the Physics Institute of the Academy of Sciences could conceivably have been considered as 'classified', thereby threatening his chances of emigration, he denied himself the possibility of creative work in his own field and was employed as an engineer. The latest Tass allegation that Shcharanskii has been organising the collection of classified information to send "abroad" thus adds a bitter twist to his story.

Vera Rich

IN BRIEF

Brain drain persists

Although the brain drain has been partially plugged by restrictive immigration regulations in the United States, it is still proceeding apace, according to a study released by the National Science Foundation (NSF). Between 1966 and 1975, some 10,000 scientists and engineers emigrated to the United States, the study indicates, though the average annual influx dropped from 11,500 to 6,500 during that period. The two chief influences were an immigration regulation adopted in 1965 which eliminated the preference previously given to European applicants for immigration, and a regulation adopted in 1971 which removed scientists and engineers from the priority list because of high unemployment among scientific workers in the United States.

The result has been an overall drop in the number of scientists and engineers entering the United States, coupled with a marked shift in the relative numbers coming from Europe and Asia.

In 1965, the ratio of scientific emigrants from Europe and Asia was 40% and 28% respectively; by 1975, it had swung to 19% and 56%. Throughout the decade, the ratio of engineers to scientists remained fairly constant at about 2 to 1.

Waste report

The UK National Radiological Protection Board (NRPB) has followed up its recent publication dealing with disposal of high-level radioactive waste on the sea-bed with the release this week of a report studying the ways low-level waste is dispersed in the ocean (NRPB-R58, HMSO, 50p). Disposal of such waste, which consists of contaminated equipment, clothing and rubbish packaged with concrete or bitumen in metal drums that eventually corrode, has gone ahead under international arrangements for some years. The report describes a two-part (short term and long term) mathematical model for estimating maximum con-

centrations of radioactivity in water for continuous disposal, and can be used to assess the ocean's limiting capacity to accept radioactive waste.

Geos switch-on

Switch-on of experiments on Geos started on 29 April. Five out of the seven experiments have so far been tried and are working; the boom deployment programme is progressing satisfactorily. Eight hours of effective data transmission per day will be possible but only about four hours will be beyond 5 earth radii where the experiments are designed to operate. At a longitude of 35° E next week Geos could interact with Northern Scandinavian ground stations, but very little useful data are expected because of the movement of the satellite. Only a few per cent of the original data-taking capabilities of the mission can be realised, putting the IMS in jeopardy. A prompt launch of the spare Geos remains desirable.

EARLY in April the Federation of American Societies for Experimental Biology met once again in Chicago. It was, as always, the windy city, with snow flurries blowing off Lake Michigan. I stayed in the Conrad Hilton Hotel, as I had in 1941, when it was the Hotel Stevens. Some years later, I think in 1956, I had squeezed my way into a doorway of a packed room to hear a paper by Volkin and Astrachan on 'DNA-like RNA'. It was one of the first premonitions of messenger RNA. Prior to this, ribosomal RNA seemed the only possible template for protein synthesis, and its uniformity of composition in different organisms was not compatible with the diversity of their proteins. This year a capacity crowd of thousands sat comfortably in a big new theatre to hear a virtuoso performance by Fred Sanger as he described the nucleotide sequence of Φ X174 containing instructions for 1,849 codons in genes for eight proteins. One of the audience told me afterwards that it was always a humbling experience to listen to a lecture by Fred.

My thoughts turned to Federation Meetings, years ago, when a livelier iris changed upon the burnished dove and sprightly young women scientists caught my bachelor eye, or I tried to catch theirs. Alas, in 1977, I found myself looking automatically at all people with grey hair because I expected to recognise them as my contemporaries; souls that have toiled, and wrought, and thought with me. Such are the dividends of advancing

years. But there are really few experiences so rewarding as that of greeting an old colleague and friend whom one has not seen for a long time.

Federation meetings

THOMAS H. JUKES

I was the final speaker in an evening session in a meeting room at the Conrad Hilton Hotel. A modern note of social disintegration was sounded by an incident that took place within five minutes after I finished; someone stole the slide projector. I never found out if the purloiner was a disgruntled scientist who sought belated revenge because his slides at some meeting had been shown upside down.

The 'poster sessions' in the

McCormick Place Exhibit Hall were obviously very successful. This format has increasingly replaced the old system of 12-minute papers read in an often crowded and usually darkened room. On Tuesday afternoon, for example, there were 13 'regular' sessions in biochemistry and 9 poster sessions. The authors are on hand at their assigned posts, complete with posters, graphs and abstracts, to discuss their work with all passers-by who are interested. This gives much more opportunity for an exchange of views than is afforded by two or three minutes allowed for questions after a regular paper. The Exhibit Hall was, as ever, the scene of a dazzling profusion of intricate biochemical equipment and apparatus. One would not have suspected any slow-down in research.

There was the usual profusion of diverse social gatherings and cocktail hours, ranging from the Annual Meeting and Wine Mixer Association for Women in Science to the Korean Biologists Tea and the Physiology of Exercise Mixer.

I am afraid I have very little sympathy with those who oppose the granting of travel funds for scientists to attend such meetings. At such gatherings, names become changed into people, ideas are exchanged and stimulated, and long friendships are started. The public has garnered sufficient dividends from the progress of the biological sciences in the past 50 years to support the continuation of the Federation Meetings.

news and views

New accelerators for high energy physics

from W. J. Willis

The super proton synchrotron (SPS) at CERN in Geneva is due to be inaugurated officially on 7 May. In this and the following two articles practicing scientists look at future prospects.

A NEW stir of activity among physicists studying elementary particles has become unmistakable. In part due to the excitement generated by the suggestive discoveries of the past 3 years, it has also to do with the sense that visions of important future facilities may become real. In January the SPS, a 400 GeV proton accelerator was commissioned for physics at the CERN site near Geneva, bringing to completion the generation of facilities planned in the 1960s. While these are exploited, discussion of possible future steps grows more serious.

Extension of existing facilities

Some important and interesting developments of the existing facilities are already underway, speeded by competition, the excitement of the new discoveries, their relatively moderate cost, and the fact that complicated institutional arrangements were not required. Two similar storage rings to study the collisions of beams of electrons and positrons at energies up to more than 20 GeV are under construction at the DESY Laboratory, near Hamburg, and at SLAC, in Stanford. The German machine, PETRA, is expected to be completed in 1979 with the Californians' PEP some months behind. The recent experiments on e^+e^- annihilation in the few GeV region led to the rapid discovery of many new particle states and showed that this is a clean and powerful way to study the creation of states containing many strongly interacting particles. The notion that a good many of the discoveries will be made quickly, and the similar schedules for the two machines, have combined to give their construction somewhat the character of a race.

Another ongoing project is the Energy Saver-Doubler at Fermilab, a characteristically bold endeavour which represents the first commitment to build

and operate a large number of superconducting magnets. These will be placed in the existing tunnel and the protons transferred to the new ring at an energy of several hundred GeV. The risk is moderated by the consideration that the project will be justified, as an Energy Saver, even if the magnetic field initially attains only the 2.2 Tesla now provided by the conventional magnets. Of course it is expected that the superconducting magnets will reach fields greater than 4 Tesla, allowing the new ring to accelerate protons to 1 TeV (1,000 GeV). The necessary strengthening of the experimental beams and shielding would then allow a new experimental programme at higher energies and perhaps intensities. The schedule and cost are not firm, but the experimental programme at 1 TeV is certainly several years distant.

These developments open the way to many new kinds of experiment, but it should be pointed out that they do not provide higher centre of mass energies than those already available from the collisions of the two 31-GeV proton beams of the CERN ISR. This falls short of the region of 100 GeV of available energy, where the weak and electromagnetic interactions may reach the same strength. The recent discovery of neutral weak currents supplies a missing link in the argument that these two interactions are different aspects of a single fundamental phenomenon. It is widely believed that when the necessary energy is reached, their unity will become apparent, probably by the creation of a new family of very massive particles which are the quanta of the weak electromagnetic field. In testimony before a Congressional committee several years ago, T. D. Lee drew an analogy between the consequences of this hoped-for unification and the unification of electrical and magnetic forces achieved by Faraday and Maxwell. In this context the PEP-PETRA race can be viewed as an exciting skirmish on the way to the goal sought for so many years.

Colliding beams and electron cooling

Attempts to reach this goal are foreseen on two levels. Both Fermilab and

CERN hope to reach centre of mass energies well above 100 GeV by colliding beam experiments based in large part on the use of existing equipment. At Fermilab, one possibility considered is to store protons in the Energy Doubler at energies up to 1 TeV, and collide them with protons in the existing Main Ring at about 200 GeV, giving centre of mass energies up to 900 GeV. Also, both laboratories are planning to continue the investigation of the technique of "electron cooling" invented and successfully demonstrated by G. Budker in the Soviet Union, to allow the storage of large currents of antiprotons. In this technique, an electron beam is passed through a beam of antiprotons created by the accelerator and captured in a small storage ring. The antiprotons are created with a spread in energy and angle which causes them to fill the aperture of the storage ring. The electron and antiproton beams, which have the same e velocity, are coupled by the Coulomb force. The antiproton beam exchanges its disordered motions with the constantly renewed electron beam, and is damped into a narrow beam occupying only a small part of the storage ring aperture. More antiprotons are injected and the process repeated over many hours, until a large current of antiprotons is built up. These are then injected into the large ring, together with a counter-rotating proton beam, and accelerated to the maximum energy. Apart from a possible advantage of antiproton-proton collisions in creating new states, the noteworthy feature of this scheme is that it requires but a single large ring, and thus allows the conversion of an existing accelerator into a colliding beams facility. This has long been the aim of Budker's group at Novosibirsk, viewed with a certain scepticism by the orthodox accelerator scientists until the recent success. A very different cooling method has also been demonstrated recently at CERN.

In the forthcoming tests, CERN has the advantage of using an existing small storage ring, but the ring Fermilab will construct for tests with protons can also be used for antiproton

accumulation in the final scheme. If all goes well, these efforts could lead to colliding beam experiments in the early 1980s. If this technique is applied to the Energy Doubler, we might see collisions with 2 TeV in the centre of mass in 5 years or so.

Plans for future facilities

These heady visions must be balanced by some cautions. Major extrapolations are involved in these designs, and the event rates which will actually be achieved are most uncertain. The experiments in the region where the weak and electromagnetic interactions become equal are not easy, and involve quite small cross sections. It may be that these projects reach success without being able to perform these difficult experiments in a conclusive manner. In any case, these are attempts to carry off a coup, and will not yield facilities where many experiments can be carried out simultaneously. The more conventional plans to reach the energy goal may prove to be the more important for physics. The discussion of major new facilities proceeds primarily within the traditional framework of the USA, the European collaboration, and the Soviet Union, but efforts to establish a world-wide collaboration have begun to have effects, mainly indirect. The "world accelerator" discussion has been carried out off and on since 1960, but achieved its most interesting stage last May in a meeting at Serpukov. The participants agreed on two points. They undertook

to avoid a needless duplication of facilities. Whether this is an admission of guilt in the PEP-PETRA case is moot, but it has real implications for the next step. It was further agreed that an attempt would be made to fix upon a world facility in about 10 years' time, leaving enough time for one more round of "regional" facilities. The International Union of Pure and Applied Physics was assigned the task of studying some organisation for further work, with US, European, Soviet, and Japanese participation. (A rapid cycling proton accelerator of 10 GeV is now working in Japan.)

The reality of the world accelerator may be uncertain, but the fixing of a kind of deadline for any large regional effort has immediate consequences. As it happens, the US has a mature proposal ready for a decision. This is ISABELLE, a superconducting proton storage accelerator of 200–300 GeV, to be built at Brookhaven, and given a good chance of gaining approval soon. Partial approval has apparently been granted at Serpukov for an accelerator of 2 TeV, called UNK, to be converted to storage rings later. In Europe, it has not been possible to carry out detailed planning for a new facility while constructing the SPS, an expensive instrument whose approval and financing has already created tensions among the CERN collaboration. This is an awkward time to seek bilateral arrangements with the US, since Brookhaven is confident of obtaining approval without further complications.

This situation, and the 10 year deadline, has led to a sense of urgency among European particle physicists. Luck seems to be with them, since the great success of the electron-positron colliding beams has revealed a big gap in the world plans, in that there has been no plan for an e^+e^- machine to reach the goal of 100 GeV or greater, though it is probably possible to build such a machine at a reasonable cost. If the neutral weak currents arise from a heavy particle with a mass of about 80 GeV, this machine would be a superb tool for investigating many new phenomena, and it is in any case a powerful complement to the proton collisions. This idea is now under study by the European Committee on Future Accelerators, and by CERN, along with the LSR, a 400 GeV proton storage ring, and other possibilities.

Ideas for the world accelerator itself are not at all definite. Most often mentioned is the VBA, a proton accelerator (storage ring?) of more than 10 TeV. Part of the enthusiasm for such a project reflects the feeling that one of the major successes of CERN has been to demonstrate that many nations can work together to achieve a common goal, even one involving deeply integrated efforts in complex technology. It would indeed be a happy result if the same success could be achieved on a world wide scale, while providing the next generation of young physicists with completely untrodden territory to explore. □

New physics and new accelerators in Europe

rom B. H. Wiik

OVER the past few years great progress has been made in our understanding of the fundamental laws of nature as revealed by high energy physics. On the one hand, there has been an explosion of experimental discoveries—neutral weak interactions, probably charmed particles, perhaps a new lepton. Obtained at the present generation of high energy accelerators—CERN, DESY, FNAL and SLAC—most of these discoveries support the idea that weak and electromagnetic interactions can be unified in a single gauge theory. These experimental discoveries have been rivalled by a similar theoretical explosion. It now seems possible to suggest that the strong nuclear forces may also be reflections of an underlying gauge theory of quarks. It is even permissible to dream of the super-unification of all elementary particle forces.

Experiment and theory in high energy physics must engage in a continuous dialogue for this progress to continue. Do new quarks exist beyond charm? Are there yet heavier leptons? What is the structure of the weak and the strong interaction? Is there a symmetry between quarks and leptons? Answers to some of these crucial questions may be forthcoming from experimental facilities which are now available or under construction. In Europe the hopes are centred on the ISR and the SPS at CERN and at DORIS and PETRA at DESY.

ISR

At the ISR—the large proton-colliding beams at CERN—a new generation of experiments specifically designed to search for new particles is under way and results are expected within a year. With impressive ease CERN recently commissioned

the SPS, a new 400 GeV proton synchrotron. The SPS supports, among other studies, extensive programmes in neutrino physics and high energy muon scattering. These lepton beams are well suited for the production of hadrons composed of new quarks and the experiments are also expected to give important clues to the nature of the strong interaction.

PETRA

The commissioning of PETRA, the high-energy e^+e^- accelerator now under construction at DESY, is little more than a year away. This storage ring will initially explore the energy range up to 30 GeV in centre-of-mass and ultimately probe even beyond 50 GeV. As has been proven with SPEAR at SLAC and DORIS at DESY, e^+e^- collisions are powerful sources of new particles, both quarks and leptons. At the highest PETRA energies the neutral

weak interaction may start to become comparable to purely electromagnetic effects.

Need for higher energies

However, there are crucial questions which can only be settled at energies well above those available with the present accelerators. From energies of a few keV to a few hundred GeV the weak interaction is well described by the point Fermi interaction resulting in a total cross section increasing linearly with energy. For the weak interaction to be consistent with unitarity, one generally assumes that it is mediated by the exchange of heavy vector bosons, to which gauge theories usually give masses between 50 GeV and 100 GeV. The simplest gauge model which relates the electromagnetic and the weak interaction predicts a charged $W^\pm$ boson with a mass around 65 GeV and a neutral Z^0 boson with a mass about 80 GeV. It is obviously crucial to find the intermediate vector bosons and to directly demonstrate that the electromagnetic and the weak interactions are simply manifestations of a single interaction. Another very important question which requires super-high energies to answer, is related to the strong interaction. If strong interactions are described by an asymptotically free gauge theory of quarks, then they should grow progressively weaker at very short distances. Short distances can only be probed at very high energies.

There have been several recent studies in Europe and elsewhere of accelerators with sufficiently high energies to confront these questions in the near future. At present, plans in Europe are centred on upgrading existing facilities at CERN. The boundary conditions are low cost and a reasonably short time-scale. Two projects which meet both these conditions take advantage of the excellent properties of the SPS to collide either antiprotons or electrons with the protons in the main ring.

Upgrading SPS

In the $p\bar{p}$ option protons and antiprotons are injected into the SPS in opposite directions and made to collide in one or perhaps two of the long straight sections. The SPS can run continuously at 270 GeV resulting in a cms energy of 540 GeV for the $p\bar{p}$ system. This option will provide a first glance at strong interactions in a new energy region where cosmic ray studies give tantalising indications of novel phenomena. It also offers the enticing possibility of directly producing the $W^\pm$ and Z^0 . Rates for these processes have been estimated using a Drell-Yan mechanism and found to be acceptable provided a luminosity of at least $10^{30} \text{ cm}^{-2} \text{ s}^{-1}$ can be reached. Recent results from the ISR on stochastic cooling and from Novosibirsk on electron

cooling—both used to increase the phase space density of the antiprotons—may make such luminosities within reach. A working group at CERN is now studying the following scheme which combines both methods. Protons from the 28 GeV proton synchrotron are used to produce antiprotons with momenta around 4 GeV/c. These are injected into a small storage ring, cooled stochastically, decelerated to 100 MeV kinetic energy and transferred to a second ring where they are further cooled by an intense electron beam travelling with the same velocity as the antiprotons. To accumulate a sufficient number of antiprotons this process is repeated many times over—in fact it takes nearly a day to accumulate the 10^{12} antiprotons needed. After accumulation, the antiprotons are transferred to the SPS and accelerated together with the protons to high energies. This is a very exciting project and a vigorous study is now under way to see if it can be realised with luminosities of $10^{30} \text{ cm}^{-2} \text{ s}^{-1}$ or above. A similar project is also under study at FNAL.

In the electron-proton option the proponents suggest injecting electrons from a small synchrotron like NINA into a large electron ring mounted in the SPS tunnel. Electrons with a maximum energy of 25 GeV to 30 GeV are made to collide with protons of energies from 140 GeV to 400 GeV during a normal or somewhat extended SPS cycle, with an intermediate flat top. Collisions can take place in two interaction regions and the peak luminosity may be of the order of $10^{32} \text{ cm}^{-2} \text{ s}^{-1}$. This scheme allows the normal SPS programme to continue and since the protons only remain for very short times in the SPS ring, all problems connected with storing a tightly bunched proton beam are minimised.

The proposed electron-proton option will extend the momentum transfer and energy range presently available with the neutrino and the muon beams at the SPS or at FNAL by nearly two orders of magnitude, probing a region where a point-like weak interaction would be stronger than the electromagnetic interaction. However, according to gauge theories the weak interaction will be damped such that both interactions will be of similar strength. The experiments will elucidate in detail how the weak and the electromagnetic interactions are unified—do only one $W^\pm$ and one Z^0 exist as presently conjectured, or is a more complicated mechanism involving many new heavy bosons responsible? Crucial information on the strong interaction can also be obtained by using virtual photons to probe the strong forces at short distances. Do they indeed weaken as the relevant distances shrink? Conclusive tests of this conjecture require momentum transfers well above those available at present accelerators.

Both the $p\bar{p}$ and the $e\bar{p}$ option will

greatly advance our understanding of nature and they are both natural upgradings of the present SPS accelerator. Detailed studies of technical feasibility, and estimates of the cost and the interference with the SPS both during construction and operation are now under way for both projects, with the aim of making a full evaluation of both options by the end of 1977.

Future accelerators

Apart from exploiting existing facilities, one might also be optimistic and hope that Europe will be able to construct a large new accelerator within the next 10 years.

What questions might then be pressing, and what sort of large new accelerator would be needed to answer them? Probably we will want to study in detail the decays and interactions of the intermediate vector bosons. Do they have the characteristic gauge couplings apparently necessary for a calculable renormalisable theory? Does the debris of $W^\pm$ and Z^0 boson decays contain as yet unknown quarks or leptons? But by that time we may hope to be probing beyond gauge theories. In all such models yet constructed, the mass differences between hadrons and leptons, and between the photon and the vector bosons, arise by breaking the gauge symmetry with the Higgs mechanism. The associated point-like scalar Higgs particles are probably very difficult to find, but may be our only window into a world beyond the gauge principle. Perhaps also the super-unification of the strong with the weak and electromagnetic interactions will be another crucial issue 10 to 15 years from now.

Many members of the European high energy community feel that these questions can best be answered by colliding electrons and positrons at very high energies. The results of first studies of such large e^+e^- rings were reviewed at a week-long working meeting organised by ECFA (European Committee for Future Accelerators) at DESY at the end of February this year (see accompanying article). This meeting created much enthusiasm among the participants and detailed feasibility studies of such an accelerator are now under way. Besides the arguments of pure physics, there are also strong political arguments for an e^+e^- ring as the next major European high energy project. These arguments are based on the fact that large proton synchrotrons and proton-proton colliding rings in the multi-TeV range are now under active consideration in both the US and the USSR. A European high energy e^+e^- ring would therefore be unique and fit well into attempts to coordinate the construction of new high energy accelerators on a world-wide basis. □

The accelerator after next

from D. J. Miller

"WHY have a conference about the accelerator after next, when we are just building the next one?" That is the question a lot of us were asking as we flew to Hamburg on 22 February for a 6-day working meeting on electron-positron storage rings of 100 GeV (the LEP, Large Electron Positron project). The organisers, ECFA—the European Committee for Future Accelerators—seemed to be looking a very long way into the future but a number of the participants argued that we should commit ourselves to building such a machine as soon as the money can be found.

The reason for their urgency lies in the present theoretical situation. Results from the present e^+e^- storage rings, from CERN and from Fermilab are giving increasingly firm and consistent support to the idea of charm and its relation to the neutral current in weak interactions. As a consequence, theorists are building more and more detailed models of elementary particles, and predicting quite specific properties for the forces which govern their interactions. The final unification of the weak, electromagnetic and strong forces has not been written down yet, but many parts of the unification scheme exist and will probably survive in something like their present form. In particular, a family of massive particles, the intermediate vector bosons ($W^\pm$ and Z^0), is required to account for the weakness of the weak interaction, and another family of 'Higgs bosons' is needed to relate the $W^\pm$ and Z^0 to the photon the carrier of the electromagnetic force. Precise predictions exist for the masses of the $W^\pm$ and Z^0 , though nobody claims that these predictions are unique. It is also expected that, as e^+e^- collision energies are increased, the weak interaction should grow in strength compared with the electromagnetic force. At the predicted mass of the Z^0 , 80 GeV/ c^2 —equivalent to 40 GeV on 40 GeV e^+e^- collisions—one of three things should happen. There could be a resonance, similar in some ways to the ψ and ψ' but even more interesting to analyse because its decays would tell us exactly how the weak neutral current interaction couples to every other kind of particle. There could be a steady increase of the weak interaction rate which would completely swamp the falling electromagnetic rate. Or there might be

a slow levelling-off of the increase in the weak interaction rate, with no resonance. Such a result would set a lot of puzzles, but their answer would come directly from a study of the events seen.

If the Z^0 resonance is seen, then it is expected that pair production of W^+ and W^- would also occur at a slightly higher energy, and at these energies it might also be possible to observe the production of Higgs bosons by way of $W^\pm$ or Z^0 intermediate states.

Some theorists are sufficiently confident in these predictions that they say we should go ahead as fast as possible and build the LEP machine. Others are more cautious. The first two 15 GeV on 15 GeV e^+e^- machines will not work until 1978 (PETRA in Hamburg) or 1979 (PEP in Stanford, California). The CERN SPS is just taking its first data. A large number of the predicted effects will begin to be seen at the 5 to 10% level with these devices. Perhaps we can get some more indirect evidence about the masses of the $W^\pm$ and Z^0 from neutrino interactions. Then there are relatively cheap advances which could be made by adding an antiproton-proton facility (270 GeV on 270 GeV) to the SPS, or by adding a 25 GeV electron ring to collide with 270 GeV SPS protons. Above all, money is tight and may be getting tighter. Who would pay for a new machine costing over one thousand million Swiss francs to build, and consuming over one hundred megawatts of power?

Electron machines are very greedy for power because the beams radiate energy away in the form of synchrotron radiation. That is why it is much easier to build high energy proton accelerators than electron accelerators. But electron-positron collisions seem to be a much better way of doing high energy physics. When protons collide at high energy the really interesting part of the interaction is between the constituent parts of each proton, the three quarks. This means that, on average, only one third of the energy of each beam is actually available in the basic quark-quark collision, compared with e^+e^- collisions where all the energy of each beam is concentrated in one particle. In addition, in proton-proton interactions, the quark-quark collision is accompanied by a splash of relatively uninteresting particles from the other two 'spectator' quarks in each

proton. The most important class of electron-positron collisions is much simpler to understand because the initial particles are completely annihilated, and the final state is produced in a well-defined way.

The ECFA working-meeting reviewed the theoretical and technical case for building an LEP machine. Possible detectors were discussed and it was clear that the cost of detectors for colliding-beam machines goes up with energy in a much gentler way than the cost of detectors for fixed-target machines such as the SPS. Perhaps the most vital discussions were on the feasibility and costs of the e^+e^- machine itself. Burt Richter from Stanford had made a preliminary investigation of the parameters of a 100 GeV on 100 GeV storage ring. He based his cost estimates on the PEP ring, but suggested that a much bigger machine might gain a considerable benefit from mass-production of components. Other accelerator designers checked his calculations and confirmed that the machine was certainly feasible, even without great advances in technology. One very desirable technical innovation was pointed out. Large numbers of radio-frequency cavity resonators are needed all round the machine to provide the accelerating field which replaces beam energy lost in synchrotron radiation. But half the energy supplied to these cavities does not go into the beam; it is dissipated by resistive losses in the cavity walls. If reliable superconducting cavities can be developed these losses could be almost eliminated. Small laboratory models have been built, but a major programme of research will be needed before production models of superconducting cavities can be designed.

The final opinion of the meeting was very positive. This is the next major accelerator project that should be proposed. It is probably not too big for Europe to build on her own, although collaboration from the USA or elsewhere might be desirable. It would give direct answers to the most important theoretical questions, whereas all other machines, existing or planned, will only give indirect answers. What must now be decided is how to match an LEP project to existing commitments and how to convince governments that the expenses will be worthwhile. □

THE presence of envelope glycoproteins of oncoviruses (the new name for oncornaviruses (Fenner *Virology* **71**, 371; 1976)) on the surface of normal cells has been known since the recognition of murine G_{IX} antigen and of chick helper factor in 1969. Since then there has been much discussion of whether the expression of endogenous viral antigens has a functional role in cellular differentiation. Certainly there is differential expression of viral antigens in different tissues and at different stages of development, but Strand, August and Jaenisch in the February issue of *Virology* (**76**, 886; 1977) conclude from studies of oncovirus gene expression in embryos of different inbred mouse strains that viral antigen synthesis is incidental to differentiation. While I agree that embryos of mice or chickens that show minimal endogenous viral gene expression appear to develop quite well, it is worth noting that feral mice and chickens (jungle fowl) do express viral antigens, so one can say at least that there is no natural selection against viral antigen synthesis. Furthermore, Chen and Vogt in the same issue of *Virology* (**76**, 740; 1977) find that oncovirus envelope glycoproteins are detectable in a variety of avian species.

In this week's *Nature*, (page 23) Elder, Jensen, Bryant and Lerner describe a more detailed study of the glycoproteins (gp 70) related to C-type oncoviruses in mice. By analysis of serotypes and of tryptic peptide maps, they can divide gp70 molecules associated with normal tissues into different groups, more or less related to gp70 specificities of virus isolates. Now most mouse strains inherit several distinct oncovirus genomes

Oncoviruses and cell membrane antigens

from Robin Weiss

and this may account for much of the polymorphism. For instance in strain 129 mice, the serum gp70 resembles that of xenotropic virus, whereas seminal fluid gp70 is more closely related to the FMR group of viruses, not previously thought to be endogenous. Some of the tissue gp70-like molecules may not be linked to oncovirus glycoproteins at all. We do not know whether oncovirus glycoproteins have evolved from cellular antigens or *vice versa*, although it is well known that oncoviruses readily recombine for this genetic marker.

If viral glycoproteins serve as differentiation markers on the surface of normal cells, one might also expect cellular membrane proteins to be assembled into virus envelopes. In fact, this has been evident since de Thé, Becker and Beard (*J. natn. Cancer Inst.* **32**, 201; 1964) demonstrated the presence of leukocyte membrane ATPase in the envelope of avian myeloblastosis virus. Indeed, Beard used this enzyme activity as a biochemical assay of virus particles. Aupoix, Huppert and Vigier (*C. r. hebdom. Acad. Sci. Paris* **282**, 1379; 1976) have demonstrated chick cellular antigens in Rous sarcoma virus particles which are inactivated by treatment with anti-chick serum plus complement, and Hecht and Summers (*J. Virol.* **19**, 833; 1976) have shown that vesicular stomatitis virus picks up H-2 antigens when grown in mouse cells. What is most intriguing, how-

ever, is the report by Bubbers and Lilly last month in *Nature* (**266**, 458; 1977) that Friend virus particles selectively incorporate H-2D^b antigen. It is interesting enough that one particular histocompatibility antigen is selectively assembled in the virus envelope but Bubbers and Lilly further point out that the H-2^b haplotype confers a relative resistance to Friend disease mediated by T-cell cytotoxicity against associative recognition of both viral and H-2^b antigens on the cell surface (Blank, Freedman & Lilly *Nature* **260**, 250; 1976). Thus the association of membrane antigens which act in Zinkernagel and Doherty's (*J. exp. Med.* **141**, 1427; 1975) 'altered-self' recognition system on the cell surface may also be reflected in the virus envelope. Lindenmann (*Biochim. biophys. Acta* **355**, 49; 1974) has argued for many years that viruses could be exploited as immunological adjuvants; complexes of viral and cellular glycoproteins in virus envelopes might be most potent immunogens.

The degree of selectivity of the assembly of H-2^b into Friend virus is a little surprising in view of the variety of unrelated glycoproteins that enveloped viruses can assemble. Perhaps this is most strikingly seen in phenotypic mixing between unrelated viruses, where the 'foreign' antigen can be detected by changes in the infectivity of the virus (Zavada *Methods in Biology*, **6**, 109; 1977). This is how Chen and Vogt (*op. cit.*) detected functional viral glycoproteins in various species of pheasant and quail. No less than seven other papers in the same issue of *Virology* report studies on the assembly and behaviour of glycoproteins in enveloped animal viruses.

Cathodoluminescence topography

from John Walker

DIAMONDS are reputed to look good in candlelight; to the scientist they look even better in electron light (cathodoluminescence—the emission of light during bombardment by electrons, as in a TV screen). Cathodoluminescence topography is a technique which has developed relatively recently and complements the older techniques of X-ray and UV topography. Scientists at the University of Bristol have recently published (Hanley, Kiflawi & Lang *Phil. Trans. R. Soc.* **284**, 329–368; 1977) their fascinating results on topo-

graphically - identifiable sources of cathodoluminescence in natural diamonds, including some fine colour photographs.

Their photographs carry a message, which as yet cannot be entirely deciphered. For example, a greenish-yellow luminescence system denoted H3, which is well-known from previous non-topographic studies, is observed at slip traces and dislocations and at 'platelets' (planar precipitates on {100} planes—see *Nature* **266**, 209; 1977). However, the usual method

of producing the H3 defect is to irradiate the diamond with electrons or neutrons and then anneal it. Now, Hanley *et al.*'s diamonds might have been irradiated by natural radioactivity—indeed some of them undoubtedly had been, as discussed below—but such damage is confined to a thin surface layer. Hence these H3 defects, lying deep within the crystal, must have been produced by other means.

It is known that the H3 defect is composed of a radiation damage product (probably a vacancy), trapped at

the 'A' form of nitrogen aggregate (probably a $\langle 111 \rangle$ -oriented substitutional nitrogen 'molecule'—see *Nature* **265**, 317; 1976). One can speculate that it could also be created without irradiation by slip in the same way as some EPR centres apparently are. In this process two adjacent $\{111\}$ planes of the crystal move relative to each others, and any unfortunate nitrogen molecules bridging the planes are sheared apart (Scherbakova *et al.* *Sov. Phys. Dokl.* **20**, 725; 1975).

Another puzzle is that H3 is not normally observed in type II diamond, whereas Hanley *et al.* detect the slip traces and the H3 luminescence in type II as well as type I regions in their specimens. One way in which these problems might be resolved is to deform plastically diamonds in the laboratory, and study their cathodoluminescence before and after.

Polarised H3 luminescence was observed from the platelets. Platelets were originally thought to be composed of nitrogen. Recent evidence suggests that they may be carbon in some interstitial arrangement, though there is still controversy on the matter, as Hanley *et al.* emphasise. On the carbon interstitial hypothesis one would have to assume that the H3 defects merely decorate the platelets rather than being an integral structural part of them, though in that case it is a little surprising that the luminescence is polarised. The uniformity of the luminescence along a platelet strongly suggests that they are an intrinsic part of the structure, and hence that the platelets do contain nitrogen. It is worth recalling that Lang's model for the platelets (*Proc. Phys. Soc.* **84**, 871; 1964) contain sets of $\langle 100 \rangle$ -oriented nitrogen 'molecules'; this similarity with the A centres may explain the H3-luminescing platelets.

Hanley *et al.* also observed infrared emission from the platelets. Kiflawi and Lang (this issue of *Nature*, page 36) have confirmed this observation and have shown that it is highly polarised, and due to a broad-band system peaking at 1.25 eV, previously known to be connected with the platelets. This also seems to be an intrinsic platelet property rather than decoration by something else.

Other luminescent systems known to involve radiation damage were detected by Hanley *et al.* They included the H4, 3H (*sic*) and 575 nm systems, and were usually present in a thin surface layer whose thickness (30 μm) corresponded very well with the expected penetration depth of α particles. Hence they were probably created by natural radioactivity.

Hanley *et al.* draw attention to the intimate relationship between optical properties and growth features, and to

the inhomogeneity of most diamonds. They strongly recommend detailed examination of specimens, preferably by X-ray topography, before any experiments in which homogeneity matters. This has proved a pitfall in the past, particularly where correlation of different properties has been involved.

Since their measurements were done at or above room temperature, and largely in the visible spectral range 1.8 to 3.2 eV, extensions of the work are awaited with interest. Lower temperatures should bring greater sensitivity in the detection and identification of luminescence, and a wider spectral range might lead to further associations between point defects and growth features or other extended defects.

Diamond is the ideal subject for cathodoluminescence topography. It is transparent and shows a great many luminescence systems in the visible and near-visible spectrum. Furthermore, the processes by which it is synthesised, both industrial and natural, are not well understood; topographic studies give us some idea of how diamonds grew and what subsequently happened to them. Whether the technique can be applied as profitably to other materials is an open question. $\square$

How aseismic ridges behave

from Peter J. Smith

As newly produced oceanic lithosphere moves away from a spreading centre it cools and contracts. The result is that the average depth to the seafloor, which is $2,500 \pm 300$ m at active oceanic ridges, gradually increases with age until it is $5,800 \pm 300$ m for lithosphere formed during the late Jurassic-early Cretaceous. But although this general relationship between depth and age applies to most oceanic lithosphere, all the oceans contain many anomalously shallow features such as plateaus, rises and aseismic ridges. Such bodies have been relatively neglected by geologists, presumably because they appear not to form part of first-order seafloor spreading theory. Yet some information is available on aseismic ridges, not least from the Deep Sea Drilling Project (DSDP) which has now sampled at least 13 of them.

In an attempt to discover whether anything can be said about the origin and subsequent behaviour of such ridges, Detrick *et al.* (*Earth planet. Sci. Lett.* **34**, 185; 1977) have now brought

all the DSDP results together. And apparently something can be said. Consider, for example, the group of major aseismic ridges comprising Ninetyeast Ridge, Chagos-Laccadive Ridge, the southeast Mascarene Plateau (all Indian Ocean), Walvis Ridge and Rio Grande Rise (south Atlantic). DSDP cores indicate that all five contain older shallow-water sediments or subaerial material (ash, for example) and that the younger sediments become progressively more deep-water and more characteristic of deposition in an open marine environment. It would seem from the sedimentary record, therefore, that these ridges formed at or near sea level and have subsequently subsided by up to several thousand metres.

But by what mechanism did they subside? To find out (or perhaps to test a preformed hypothesis?) Detrick and his colleagues have determined, for each core which penetrated the volcanic basement, the age of the basement (usually from the palaeontological age of the basal sediments) and its depth corrected for the isostatic loading of the overlying sediments. When these ages and depths are plotted together, most of the points are found to lie in a curved band which bears a remarkable resemblance to the curve of age against the depth to be expected on the assumption that ridges subside at the same rate as normal oceanic crust. The agreement is not exact, for in addition to the scatter of points there is a tendency for the measured subsidence to be a few hundred metres less than that predicted. Nevertheless, it is clear that all five ridges have subsided at a rate comparable to that of normal oceanic crust.

That being the case, Detrick *et al.* propose a simple model in which an aseismic ridge forms at a spreading centre by excessive volcanism which builds the ridges up to sea level. By analogy with large-scale shield volcanism, the construction of the ridge is taken to be fairly rapid (perhaps 0.5–1.5 Myr) and the isostatic adjustment of the crust to the new ridge is assumed to occur almost simultaneously with the volcanism. Then all that happens is that the spreading, subsiding lithosphere carries the ridge along and down with it. In other words, the subsidence of an aseismic ridge is a purely thermal effect associated with the cooling and contraction of the lithosphere upon which the ridge is built.

Of course, real ridges do not often conform exactly with the model. At formation they may lie slightly above or below sea level; and since their tops are seldom if ever perfectly horizontal planes, there will be variations in level even within a single ridge. Moreover, a ridge may form slightly off the spreading axis, giving it an age rather younger

than that of the surrounding crust; and even a small deviation from the axis can produce a significant effect here because the depth-age curve close to a spreading centre is steep. Then for a ridge formed subaerially, the amount of subsidence occurring before the ridge top sank below sea level will not be recorded in the measured subsidence. The net result of all such effects will be to produce a scatter of points along the depth-age curve and also to bias the points towards too small a measured subsidence.

In the circumstances, then, it is perhaps surprising that the difference between the measured subsidence and the subsidence predicted from the behaviour of normal crust is generally no more than 300–400 m. However, such relatively small deviations apply only to the five ridges under consideration; other aseismic ridges conform much less well. For example, the Iceland–Faroe Ridge evidently formed above sea level and remained that way for at least 15 Myr before beginning its subsidence. On the other hand, Coiba Ridge, Carnegie Ridge and Cocos Ridge (Pacific) seem to have formed well below sea level and are thus now unusually deep. Then again, Madagascar Ridge, Broken Ridge and the Naturaliste Plateau (Indian Ocean) seem to have complicated histories of subsidence and uplift.

In view of these exceptions, it seems that the Detrick model can make no claim to widespread application. Yet there is more to it than that. It could be said, at least as an hypothesis, that the model applies generally to all aseismic ridges that form at spreading centres and at sea level. It does not necessarily follow from this, however, that all aseismic ridges are formed at spreading centres and at sea level. What may be true nevertheless is that, wherever a ridge forms, when it subsides it does so at a rate comparable to that of normal oceanic crust and as a direct result of the cooling and contraction of the lithosphere. □

New twists in bacterial flagella

from Michael Spencer

SINCE the last comment in these columns (*Nature*, 263, 370; 1976) more light has been shed on a number of problems relating to the structure of bacterial flagella. The first is the geometrical puzzle set by bacteria having more than one flagellum: if all are helices undergoing real or apparent rotation, can they avoid tangling together? It is a simple matter to test

this empirically by twiddling two pieces of wire between the finger and thumb, but a rigorous analysis is more daunting. Macnab (*Proc. natn. Acad. Sci. U.S.A.* 74, 221; 1977) has now tackled the problem using geometrical and hydrodynamic theory, assisted by working models.

His conclusions confirm the finger-and-thumb analysis, showing that if a group of left-handed helices starts to rotate in a counterclockwise sense when viewed looking towards the basal points of attachment, the flagella are constrained to rotate in phase with each other and will continue to do so without hindrance; they do, however, undergo some distortion so as to form a compact bundle. This is what is observed in the normal swimming of bacteria. When the direction of rotation reverses, however, the helices should rapidly interlock so as to prevent further rotation (unless the flagella are very short, as occurs in some species). If both the handedness of the helix and the sense of rotation are reversed, jamming is not expected and normal forward motion can be resumed, with the flagella pointing to the rear.

Now comes the surprising part. Macnab and Ornston (*J. molec. Biol.* 112, 1; 1977) report that a bacterium does not simply stop when the flagella reverse their rotation: the bundle flies apart, and the clockwise-rotating flagella are observed to have the smaller pitch characteristic of the 'curly' mutant. As first shown by Shimada *et al.* (*Nature* 254, 332; 1975), this has a right-handed helix. If the process is slowed down by irradiating with blue light in a high-viscosity medium these flagella sometimes form a curly bundle, giving forward motion again. Normally, however, there is a brief period of 'tumbling' followed by reversion to the previous state: a bundle of left-handed helices is reformed and the organism swims off in a new direction.

This periodic change of direction is an essential part of the chemotactic response, for external stimuli seem to influence the probability of tumbling: movement in a favourable direction suppresses the urge to tumble. This applies whether the bacteria are moving towards an attractant or away from a repellent, and must involve some kind of memory mechanism linked to the receptors (Macnab & Koshland *J. molec. Biol.* 84, 399; 1974). The observed change of helix parameters seems to be essential, for without it the bacteria might simply stop and start again without changing direction, and there would be no point in reversing the sense of rotation. Only in certain special cases can backward motion be

sustained.

The obvious question is "What makes the helix change when rotation is reversed?" After studying a number of mutant forms and the effects of abnormal growth media, Macnab and Ornston conclude that the torsional stress arising from a reversal of rotation actually induces a change in the packing of the flagellar subunits, so that the structure flips from a left-handed to a right-handed helix. On removal of the stress, the structure clicks back into its normal shape. Their paper includes a number of photographs in which the transition is caught propagating out from the base of the flagellum, where torsion is expected to be greatest. As confirmation of this idea, abnormal curly flagella are observed to flip into the normal form under stress—the reverse of the normal process.

An outstanding problem—apart from the key question of how rotation is generated—is the nature of the forces that hold the subunits of a flagellum together. Kamiya and Asakura (*J. molec. Biol.* 108, 513; 1977) have some new evidence which supports the idea that electrostatic charge interactions are important. It has been known for some time that reconstituted flagella from *Salmonella* can change from the left-handed normal helix to the right-handed curly form on reduction of the pH. Kamiya and Asakura now report that the same transition can be induced by an increase of pH above neutrality. In this case, as for the acidic transition, the curly form is attained by passing through an intermediate ('coiled') form. It can hardly be a coincidence that this sequence of transitions is just what would be expected from a geometrical packing model of the kind suggested by Calladine (*J. theor. Biol.* 57, 469; 1976). It looks as if the ionisation induced by extremes of pH causes longitudinal rows of subunits to rearrange themselves, causing a discrete change in the helix parameters for each row thus transformed. Calladine's working hypothesis of a 'hinge' between subunits, giving two alternative arrangements, is supported by several biological examples; the latest to be described is tomato bushy stunt virus (Winkler *et al.* *Nature*, 265, 509; 1977).

This latest work on flagella illustrates how electrostatic and mechanical forces can separately induce a similar structural change. Macnab and Ornston make the point that a stress-induced transition may be relevant in other systems having regularly-arranged subunits. Who knows, at present, whether it might not be at the heart of muscular contraction? But that is another story. □

Wanted alive—but handle with care

PHYSIOLOGY laboratories in Britain, Germany and the United States are urgently seeking suppliers of the tropical giant water bugs of the genus *Lethocerus*, whose flight muscle is one of the most important preparations used in the study of the molecular mechanisms of contraction in insect flight muscle.

During a symposium held recently in Oxford, UK, the problem of obtaining living material from tropical countries and the lack of success in breeding these bugs in the laboratory were discussed. The laboratories shown in the table are all now in the market for the living insects; the table shows approximate requirements and acceptable species. They would be glad to hear from anyone who can provide these insects and would be willing to make contracts with reliable suppliers. Transit costs would be refunded and all prices are quoted in US dollars.

These giant bugs are aquatic but are easily collected when they fly to lights in damp weather—in the rainy season now approaching in many of the countries where they are found. Provided they are individually restrained in envelopes with an adequate air supply, up to 50 can be packed in a perforated tin and sent by air freight. These insects have a poisonous bite and should be handled with care.

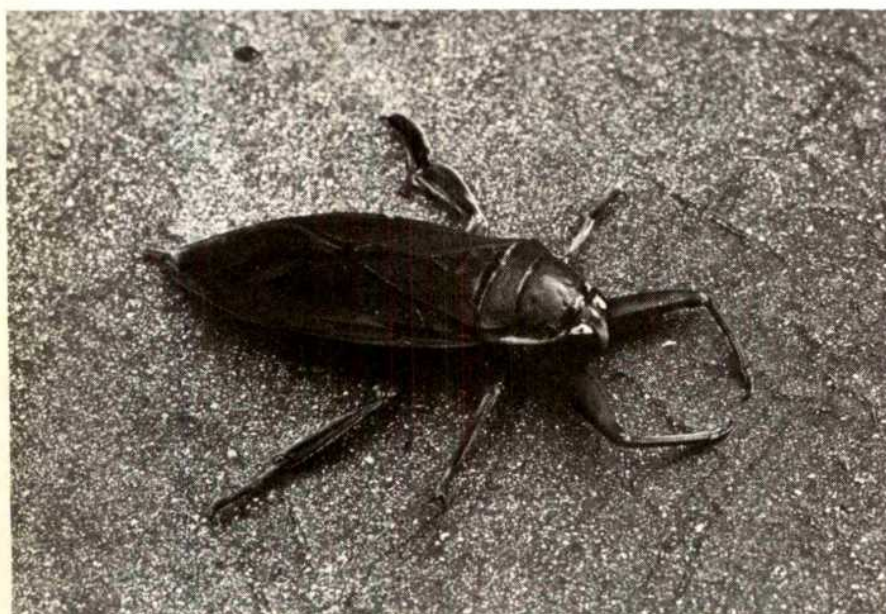
The proceedings of the symposium will be published by Elsevier-North Holland Biomedical Press later this year, entitled *Insect Flight Muscle*.

Address	Regular monthly requirement	Maximum requirement for irregular supply	Acceptable species
Professor J. W. S. Pringle, ARC Unit, Dept of Zoology, Oxford OX1 3PS, UK	20 at \$5 each	3,000 at \$0.5 each	A + B
Dr D. C. S. White, Dept of Biology, The University, York, UK	4 at \$5 each	120 at \$0.5 each	A + B + C
Professor J. C. Rüegg, Universität Heidelberg, II. Physiologisches Institut, Im Neuenheimer Feld 326, 6900 Heidelberg, FRG	1 at \$10 each	2,000 at \$0.5 each	A
Dr K. C. Holmes, Max-Planck-Institut für Medizinische Forschung, 6900 Heidelberg, Jahnstrasse 29, FRG	2 at \$10 each	250 at \$2 each	A
Dr M. K. Reedy, Dept of Anatomy, Duke University Medical Center, Durham, North Carolina 27710, USA		Special arrangements	
Dr J. Squire, Biopolymer Group Dept of Metallurgy and Materials Science, Imperial College London SW7 2BP, UK		Special arrangements	
Dr G. Steiger, Los Angeles County Heart Lab., Center for Health Sciences, UCLA, California 90024, USA		Special arrangements	

A = *L. maximus* (Venezuela, Trinidad); *L. indicus* (India, Malaysia, Indonesia); *L. colossicus* (Mexico).

B = *L. cordofanus* (Africa, South East Europe); *L. annulipes* (Guyana, West Indies); *L. insulanus* (Australasia); *L. griseus* (Florida, Mexico).

C = *L. uhleri* (South-East United States).



Lethoceros: \$5,000 market



A hundred years ago

M. HENRY GIFFARD is constructing, near the Champ de Mars at Paris, a workshop for the preparation of sulphate of iron. The apparatus was tried for the first time last Friday, when the balloon *Eole* was inflated in an hour and a half, and was sent up with an aeronaut. The capacity of the balloon being 220 cubic metres, the rate of production is very satisfactory. It is expected that the sale of sulphate will cover almost all the expenses, so that numerous scientific ascents may be made in the ensuing summer. The monster captive balloon of 20,000 cubic metres will be inflated by the same process.

from *Nature* 16, 3 May, 17; 1877.

articles

Evidence of Precambrian continent–continent collision in Western Norway

Erling J. Krogh

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Norwegian gneiss eclogites are formed in situ within the temperature–pressure range 300–800 °C, 8–22 kbar. The regional $K_{D(FeO/MgO)}^{ga+cpx}$ distribution pattern indicates an increasing P–T trend towards the coastline. Retrograde reactions indicate a nearly isothermal uplift history. A plate tectonic model, involving continent–continent collision and subduction is proposed for the formation of these eclogites.

ECLOGITES commonly occur as bands and lenses within the West Norwegian gneiss region. The surrounding rocks are mica-rich schists, biotite–plagioclase gneisses and biotite–two–feldspar gneisses^{1–4}. They are assumed to be metamorphic equivalents of original supracrustal rocks. The metamorphism of these gneisses lies in the almandine amphibolite facies, but eclogites are also found within a complex of granulite facies rocks².

K–Ar ages on hornblends from the eclogites give 1,750–1,850 and 980–1,170 Myr, and micas give 370–415 Myr (ref. 5). A three point K–Ar isochron based on data from McDougall and Green⁶ (sample no. 756) gives ~940 Myr (W. L. Griffin, personal communication). Rb/Sr whole rock datings on the surrounding gneisses give ~1,700 Myr (refs 4, 6) and ~1,000 Myr (ref. 7), while mineral Rb/Sr (ref. 7) and U/Pb (ref. 8) data indicate a Caledonian effect. These datings suggest that the eclogites are of the same age, and have the same crustal history as the surrounding gneisses.

The formation of Norwegian eclogites has, however, long been a controversial subject. O'Hara and Mercy⁹ suggest that the eclogites were emplaced into the gneisses as wedges of crystalline rocks that were derived from the upper mantle. Lappin¹⁰ believes that the eclogites were brought to near-surface conditions by an upwelling mantle (for example a mid oceanic ridge), and taken along with a moving oceanic lithosphere plate to a subduction zone. As the subducted lithosphere went downwards, the eclogites were mechanically mixed in with the overlying continental material, where we find them today. The main argument for a mantle origin of the eclogites is that the P–T conditions necessary for eclogite formation are not realised within the crust^{9,10}. Lappin¹⁰ further argues that the maximum pressure during the metamorphism of the gneisses did not exceed 10 kb, a "limiting pressure assigned to amphibolite facies by various authorities".

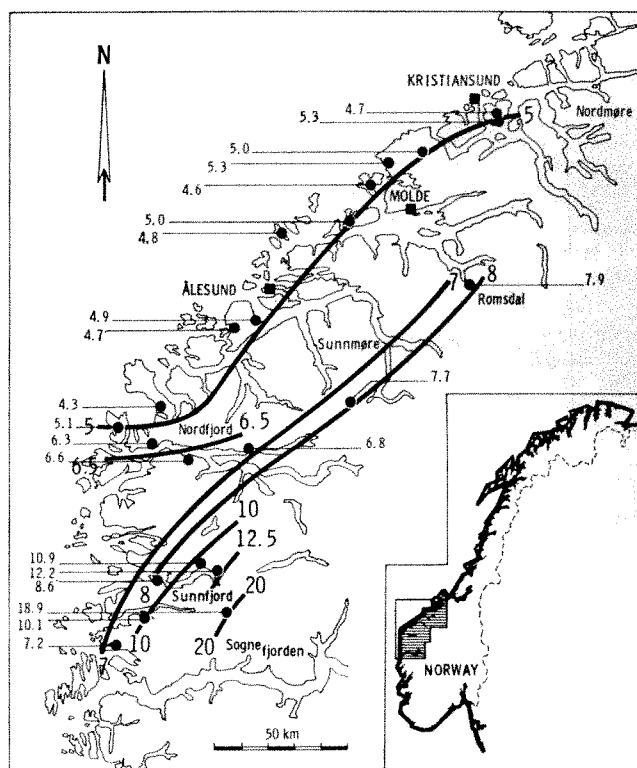
Bryhni² argues from field and structural data that although some of the eclogitic bodies are tectonic inclusions, they have not been transported over long distances relative to the enclosing gneiss. Most of the eclogites, however, form concordant lenses or bands parallel to the foliation of the gneiss. There is structural and petrological evidence for the formation of eclogites *in situ* together with the enclosing gneisses^{3,4,11–14}. Griffin and Råheim¹² describe dolerites that are partly altered through coronites to eclogites, and show that they were formed *in situ* at the same P–T conditions as the eclogites in the same

area. This implies that eclogites may be formed within the crust.

I have recently discovered blueschist facies mineralogy in eclogites from the Sunnfjord area, also indicating a crustal origin of these rocks.

Samples from more than 30 different localities (Fig. 1) were chosen for a detailed study of the Norwegian gneiss eclogites. The general primary mineral assemblage in the eclogites from Sunnfjord area is garnet + omphacite ± phengite ± paragonite + barroisite/glaucophane + quartz + rutile ± kyanite ± zoisite. In the Nordfjord, Sunnmøre and Nordmøre areas, the general primary mineral assemblage is garnet + omphacite + amphibole ± phengite ± quartz + rutile ± kyanite ± zoisite. In these areas orthopyroxene bearing eclogites are also fairly common. The primary mineral assemblage in these rocks is garnet + clinopyroxene + orthopyroxene ± amphibole ± quartz + rutile. Primary plagioclase is not present in any of the examined samples, except from albite inclusions in garnet in

Fig. 1 Locations of analysed ga+cpx pairs from eclogites, with minimum $K_{D(FeO/MgO)}^{ga+cpx}$ values. The iso- K_D -lines show a regular pattern, with K_D decreasing towards the coastline. Some of the given K_D values are mean values from several small bodies within the same area.



some few cases. Secondary plagioclase is, however, observed both together with secondary orthopyroxene as a breakdown product of quartz + garnet, and as symplectitic intergrowths with secondary diopside or hornblende. These secondary features are most common in the NW part of the area.

Experimental work has shown that the distribution of Fe^{2+} and Mg between garnet and a co-existing FeMg-mineral is a function of temperature and pressure¹³. If the distribution coefficient is defined as

$$K_D = \frac{(\text{FeO/MgO})^{\text{ga}}}{(\text{FeO/MgO})^{\text{FeMg-min}}}$$

then the K_D value at constant P will decrease with increasing temperature, and vice versa. Similarly, the Al_2O_3 content of orthopyroxene coexisting with garnet has also shown to be a function of T and P (refs 15–19). At constant P the Al_2O_3 content in orthopyroxene will increase with increasing T .

To calculate pressures and temperatures of formation for the eclogites, EMP analyses of coexisting ferromagnesian minerals were done. Special attention was paid to the chemical zoning of the minerals.

Ferric iron was calculated assuming stoichiometry. This method seems to give very consistent results, and EMP analyses of five samples from one eclogite body in Sunnfjord give a variation in $K_D^{\text{ga+cpx}}(\text{FeO/MgO})$ of 12.6 to 13.4. Assuming an analytical error in SiO_2 of $\pm 1\%$ by weight, this will result in a maximum variation in K_D in one of these samples of 10.9 to 13.8, which corresponds to a temperature difference of only 40 °C.

The methods used for determination of P and T are those worked out by Wood¹⁶ (Al_2O_3 in opx), Råheim and Green¹³ ($K_D^{\text{ga+cpx}}(\text{FeO/MgO})$), and Krogh and Råheim ($K_D^{\text{ga+ph}}(\text{FeO/MgO})$; work in preparation). The presence of either ga+cpx+ph or ga+cpx+opx will thus allow simultaneous solutions of two equations to give unique values of T and P . If both phengite and orthopyroxene are missing, the $K_D^{\text{ga+cpx}}$ (ref. 14) can be combined with experimental and empirical work on the jadeite content of clinopyroxene^{20,21} to give P – T estimates. This will, however, only give minimum values of both P and T since there is no co-existing plagioclase to saturate the clinopyroxene with regard to jadeite.

The P – T dependance of $K_D^{\text{ga+FeMg-min}}$ and the Al_2O_3 content in orthopyroxene coexisting with garnet, can also be used to interpret the chemical zoning of co-existing minerals

with regard to changing P – T conditions. In almost all the eclogites examined here, zoning of the ferromagnesian minerals is a common feature. The zoning is most prominent in the garnets, but zoning is also observed in the other ferromagnesian minerals. The most common type of zoning, which occur in the Sunnfjord and parts of the Nordfjord area, is that in which the FeO/MgO ratio in clinopyroxene and phengite increases from core to rim, and the same ratio in the coexisting garnet, and thus the $K_D^{\text{ga+cpx}}(\text{FeO/MgO})$ decreases in the same direction. In the coexisting orthopyroxene, the Al_2O_3 content increases slightly from core to rim. According to experimental studies this reflects an increase in both T and P , from core to rim. The reversed zoning is observed in the NW parts of the gneiss region (Fig. 1), except that the Al_2O_3 content in orthopyroxene increases strongly from core to rim. This zoning reflects a decrease in both T and P . This reversed zoning is also observed in the outermost rim of normal zoned minerals. In metamorphic rocks, however, only the rims of adjacent grains can be said to represent equilibrium conditions. Whether or not cores of coexisting minerals were in equilibrium with each other at an early stage of the metamorphism is often hard to evaluate. In the cases where, for example, clinopyroxene occurs as inclusions in the garnet core, this equilibrium seems to be obvious. In these cases $K_D^{\text{ga+cpx}}(\text{core})$ is constant, regardless of whether one uses analyses of cores of separate grains, or analyses of the inclusions and the adjacent garnet core. In these rocks $K_D(\text{core})$ should give P – T conditions appropriate to an earlier stage of the metamorphism. A good example of this, taken from an eclogite with prograde zoned minerals, is as follows (sample IB12, Nordfjord):

$$K_D^{\text{ga+cpx}}(\text{core}) = 8.6; K_D^{\text{ga+cpx}}(\text{incl}) = 8.6; K_D^{\text{ga+cpx}}(\text{rim}) = 6.8$$

This very clearly shows that the $K_D(\text{incl})$, which is taken close to the garnet core, gives the same K_D as the $K_D(\text{core})$, while $K_D(\text{rim})$ of adjacent grains gives the lowest value. The Jd -content of the clinopyroxene is rather constant (43%), and this gives the following temperatures and pressures: core and inclusion: 645 °C, 15.0 kbar; rim: 700 °C, 16.0 kbar. This suggests that the minerals grew during an increase in both P and T , that is, prograde metamorphism. In other cases, however, amphibole and/or phengite, rather than clinopyroxene, occur as inclusions in the garnet core. The garnet cores were thus not obviously in equilibrium with a clinopyroxene, but clearly coexisted with amphibole and/or phengite. This is commonly the case in the southernmost part of the area (Fig. 1). Therefore one should not use $K_D^{\text{ga+cpx}}(\text{core})$ in such cases, but rather $K_D^{\text{ga+ph}}(\text{core})$. A more detailed discussion of this problem is given elsewhere (E. J. K. and Råheim, in preparation).

The calculated P – T conditions define a path of metamorphic evolution for the Norwegian gneiss eclogites (Fig. 2). The prograde part of this curve extends from ~ 8 kbar, 300 °C to ~ 22 kbar, 800 °C. This is consistent with the trend for formation of eclogites given by Råheim and Green²².

From about 800 °C there is a drop in pressure and temperature which defines the retrograde trend. From Fig. 2 it is further evident that normal zoning occurs up to 700–750 °C. Above that temperature reversed zoning occurs.

The normal zoning of the minerals is interpreted as the result of crystal growth during prograde metamorphism; the inner parts of the mineral grains failed to re-equilibrate during the growth^{14,22}. The normal zoning could thus be denoted prograde zoning.

At a certain temperature, the diffusion rates will be high enough to homogenise the minerals, and thus wipe out the prograde zoning. By cooling and uplift, the rims of adjacent grains will continue to re-equilibrate by diffusion until the system again reaches the actual threshold temperature for diffusion. Good evidence for this is recorded in the normal (prograde) zoned minerals that show a thin zone of reversed (retrograde) zoning at the rims. Råheim and Green²² also describe these features, and they reach the same conclusions.

Fig. 2 Pressure and temperature calculations, based on co-existing mineral pairs from West Norwegian gneiss eclogites, define a prograde trend from ~ 300 °C, 8 kbar to ~ 800 °C, 22 kbar (I), and a retrograde trend from ~ 800 °C, 22 kbar to ~ 700 °C, 5 kbar (II). 1, 2, Melting curves for muscovite granite with excess H_2O , and dry muscovite granite, respectively²⁵. 3, Albite $\rightleftharpoons$ jadeite + quartz²⁰. 4, Eclogite $\rightleftharpoons$ granulite transition²³. 5, 6, 7, Kyanite/andalusite: andalusite/sillimanite and kyanite/sillimanite transitions, respectively^{26,27}.

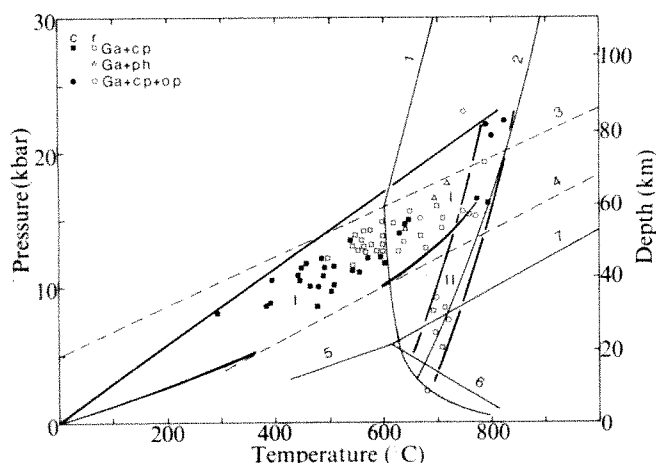


Table 1 Mineral zoning (K_D^{ss+cpx}) and calculated pressure-temperature conditions in orthopyroxene-free and orthopyroxene-bearing eclogites

	Nordfjord, prograde		Sunnmøre, retrograde	
	Opx-bearing	Opx-free	Opx-bearing	Opx-free
$K_D^{(core)}$	19.0	15.7	5.0	5.0
$P: T_{(core)}$	10.2 kbar, 480 °C	9.6 kbar, 505 °C	22.3 kbar, 825 °C	19.3 kbar, 790 °C
$K_D^{(rim)}$	6.8	6.6	5.2	—
$P: T_{(rim)}$	23.0 kbar, 750 °C	17.6 kbar, 720 °C	6.6 kbar, 700 °C	—

The appearance of the secondary granulite facies assemblage orthopyroxene + plagioclase in the rocks showing retrograde zoning reflects the retrograde trend entering the granulite facies stability field²³ (Fig. 2). The very strong symplectitisation of primary omphacite in these rocks also suggests strongly decreasing pressure. Mysen and Griffin²⁴, however, give a more detailed discussion of this problem. The regional minimum K_D^{ss+cpz} (Fig. 1), and thus the P - T pattern (Fig. 2) indicates an increase in maximum P and T from south to north-west.

It is also of interest to point out the similarities in mineral zoning, K_D^{ss+cpz} , and thus the calculated P - T conditions, between orthopyroxene-bearing and orthopyroxene-free eclogites from the same areas. This is consistent both in the prograde and in the retrograde areas (Table 1).

Bearing in mind that the Jd -contents of clinopyroxenes in these eclogites only give minimum P - T values, both the zoning of the minerals (recorded in K_D variation from core to rim), and the minimum K_D values strongly indicate the same metamorphic history for these two types of eclogites.

The P - T path shown in Fig. 2 is consistent with that expected for regional metamorphic terranes now exposed at the surface; first a prograde (burial and heating) and then a retrograde (uplift and cooling) history. It is also evident from Fig. 2 that the rocks from a relative high dP/dT were exposed to a lower dP/dT environment. Thus there must have been a considerable change in the regional geothermal gradient in the area during the metamorphic cycle.

During subduction of a cold slab, the isotherms will be depressed. Once the subduction ceases, and during the succeeding uplift, the anomalous isothermal pattern will be smoothened out, and the area restored to its normal geothermal gradient. The subduction + uplift process can thus explain the metamorphic cycle that is reflected in the zoning of the minerals of the west Norwegian eclogites.

As mentioned above, datings suggest that eclogites are parts of a continental mass metamorphosed 1,700 Myr BP, and later disturbed at ~1,000 and ~400 Myr BP. The convergent margins in this case must therefore have been of a continent-continent collision type, followed by a subduction of one of the continents under the other. Before a continent-continent collision, a subduction of oceanic crust under one of the continents would be expected, thus producing an Andes type orogenic belt. This will cause an increase in the thickness of the continental crust at the continental margin. The continental-

continental collision itself will also lead to a further thickening of the continental crusts. By a further subduction of one of the continents on the cold side of an Andes type mountain chain, the subducted continental slab may be exposed to depths of 80–100 km without significant melting (Fig. 2). This type of orogene-forming process will also lead to a high degree of isostatic disequilibrium. This implies a rapid isostatic uplift, high reliefs and a rapid erosion rate, that is, a relatively rapid decrease in pressure.

The present data give good evidence to suggesting that plate tectonics were active as early as 1,850–1,700 Myr ago. Evidence for the existence of an early (> 1,850 Myr ago) proto-Atlantic ocean of the Pacific type is also found further north along the same coastal margin in the Lofoten-Vesterålen area (E. J. K., in preparation).

I conclude that all the eclogites examined here can represent crustal rocks metamorphosed *in situ* during subduction of a continental crust to depths of 65–80 km. At these depths some mantle material may have been picked up, but the separation and recognition of the mantle derived rocks is not straightforward at present.

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Conformational change theory for auxin structure-activity relationships

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An hypothesis is advanced that the hormonal activity attributable to the heterogeneous group of plant growth compounds called auxins is a direct result of the ability of the bound auxin to undergo a simultaneous conformational change or reorientation with the receptor, while in the binding site.

THE structure of the plant growth hormone indol-3-yl-acetic acid (IAA, natural auxin) was established in 1934¹, and considerable effort has been devoted to elucidation of molecular requirements regarding auxin structure and hormonal activity^{2–6}, but as yet no theory has predicted comprehensively the relationship between molecular structure and hormonal activity.

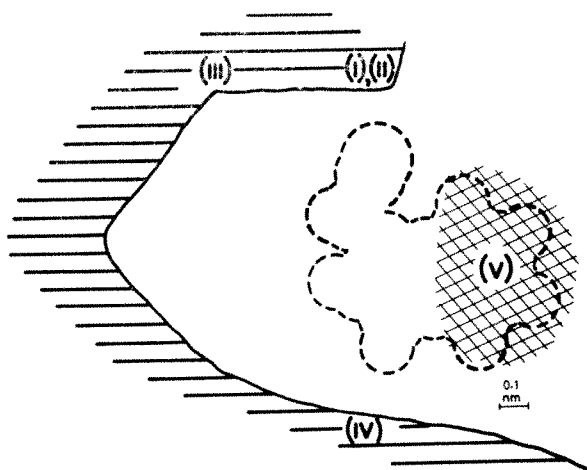


Fig. 1 Cross-sectional representation of the proposed IAA-receptor site, showing the outline of IAA (broken lines) bound in the recognition conformation. Two electropositive regions (i) and (ii) are bisected by the plane of the section; region (iii) is electro-positive, whereas (iv) is electronegative. The cross-hatching for region (v) represents areas of hydrophobic interaction with one or both sides of the receptor cleft, which has proportions resembling those of a canopied crib.

In the hypothesis described here, the hormonal activity of the heterogeneous group of compounds called auxins⁷ is attributed to the ability of the bound ligand to undergo a simultaneous conformational change or reorientation with its receptor while in the binding site of that receptor. I propose that there is a single site for primary auxin action, possessing five regions of major discriminatory significance with regard to auxin binding (Fig. 1): (i) and (ii) are two electropositive regions, possibly paired, and are responsible for recognition of the carboxyl or other acidic group of the auxin; they also show the essential conformational change relative to the ligand and remainder of the receptor; (iii), a third adjacent electropositive region; (iv), an electronegative region on, or comprising the 'floor' of, the receptor site, normally responsible for hydrogen-bonding with the pyrrole nitrogen region of IAA; and (v) an hydrophobic cleft, interacting chiefly with the benzene ring region of the indole nucleus, rather than the entire ring. Thus the site has proportions resembling a canopied crib, three electropositive regions comprising the canopy, a fourth electronegative region delimiting the 'floor', with the hydrophobic regions as the sides. The site is open for auxin recognition and binding at the 'top' and 'front'.

Recognition of IAA by the electropositive regions (i) and (ii), and subsequent binding in the site, occur with the side chain of IAA in the plane of the ring such that the oxygens of the carboxyl group lie astride the ring plane, directed over the hydrogen of carbon 4 of the indole ring; this is termed the recognition conformation (Fig. 2). The bound IAA molecule now undergoes a simultaneous conformational change with the receptor; while still interacting with regions (i) and (ii) of the site, the carboxyl group and side chain of IAA assume a position perpendicular to the plane of the indole ring, such that the oxygens of the carboxyl group are aligned so as to be co-linear with an imaginary line joining carbon 3 and nitrogen of the indole nucleus; this is termed the modulation conformation (Fig. 2).

Thus, competence as an auxin requires adoption of the essentially planar recognition conformation, and an ability to move in concert with the receptor to the modulation conformation. The obvious corollary is that molecules which can bind in the site, but which are unable to show a simultaneous conformational change with the receptor, will not

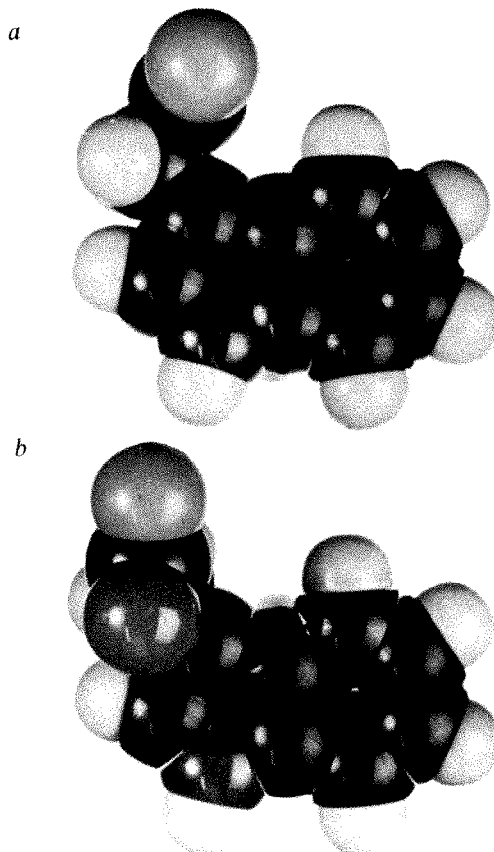
be active as auxins. By occupying the site ineffectually, such compounds will antagonise auxin action; the more potent auxin antagonists are proposed to have these properties of high affinity, zero capacity auxins.

To relate structural analogues of IAA back to this model, primary emphasis must be placed on the orientation of the side chain and carboxyl group of the test compound. These must be aligned so that their angle of inclination coincides as nearly as possible with that of IAA in its recognition conformation. Figures 3 and 5 illustrate this. With this reference point established, molecular comparisons among the whole family of auxins can be made, according to the simplistic approach that the role of a synthetic auxin is to reproduce as best as possible the features of IAA, especially its dimensions.

The exceptional molecular structure of auxins of the arylcarboxylic acid type necessitates elaborated discussion of their structure-activity relationships, but the activities of other synthetic auxins (including dithiocarbamates and acyclic analogues) can be assessed largely in terms of three factors: (1) hindrance to side chain mobility resulting from *ortho* substitutions about the side chain; (2) hindrance to side chain mobility resulting from substitutions on the side chain, and (3) hindrance to binding due to substituents in positions corresponding to substitutions on C₇, C_{7a}, or N₁ of IAA.

Factors (1) and (2) relate to the ability of auxins to show the required conformational change, that is their capacity to function; factor (1) is best exemplified by the influence of di-*ortho* substitutions in phenoxyacetic acid derivatives, where hormonal activity is markedly reduced⁸⁻¹⁰, or lost⁹⁻¹¹

Fig. 2 IAA, with side chain in the recognition conformation (a). Oxygens are astride the ring plane. In the modulation conformation (b) the side chain moves to a position perpendicular to the ring plane, and the carboxyl group realigns as shown.



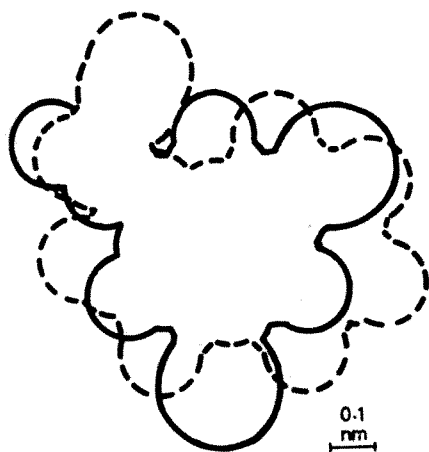


Fig. 3 Comparison of the molecular dimensions of IAA (broken lines) and 3,5-dichlorophenoxyacetic acid (solid lines), both in the recognition conformation.

unless the size of one of the *ortho* substituents does not exceed that of fluorine¹⁰. The greatly diminished activities for 2-methyl-IAA¹²⁻¹⁴, 8-chloronaphth-1-yl-acetic acid^{15,16} and 1,3-dichloronaphth-2-yloxy-acetic acid¹⁷, by comparison with their unsubstituted parent acids, further illustrate the point. The variable behaviour of naphth-1-yloxy-acetic acid in different bioassays⁴ is also explained on the basis of the rotational degeneracy of the side chain; extensive side chain movement is impeded by the *ortho* hydrogen at carbon 2 of the ring, so that any capacity for hormonal activity may be reduced to zero, with antagonistic properties arising^{18,19}, according to the degree of movement demanded by the receptor site in question. The same does not apply to the structural isomer naphth-2-yloxy-acetic acid, an auxin of moderate activity^{8,17-19}; the relocation of its side chain on the ring leaves the required movement sterically unhindered.

Factor (2) relates especially to optical isomerism in auxins. Two compounds differing only in their arrangement of groups around an asymmetric carbon in the side chain can have profoundly different activities as auxins. Not surprisingly, the absolute configuration of such enantiomers and their respective hormonal activities are very closely correlated; in all but a very few instances (discussed in detail in ref. 20) enantiomers with the *D*-configuration possess higher auxin activity than the corresponding *L*-forms, while the *L*-forms are often inactive as auxins or are auxin antagonists⁵.

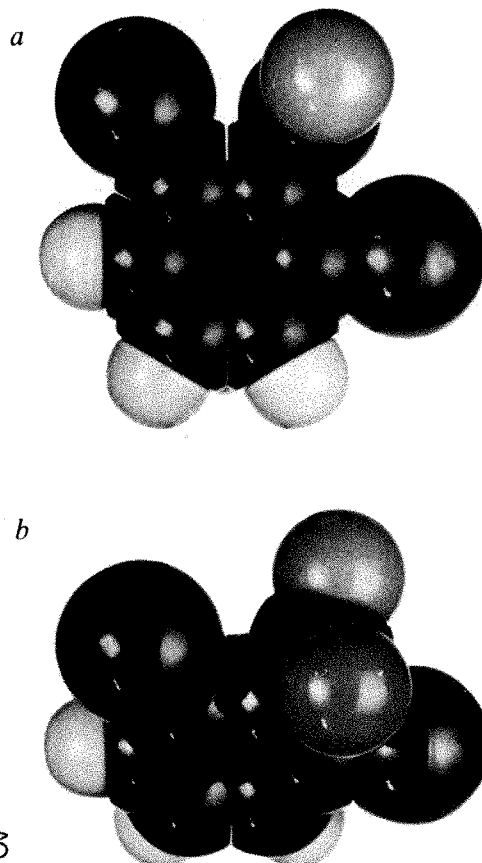
I propose that the basis of the differing properties of enantiomers is a single fundamental feature—the need to retain the ability to undergo a conformational change if capacity as an auxin is to be retained. Adsorption into the receptor site immediately imparts a sidedness on the auxin molecule, hitherto non-existent in most cases. Because the conformational change of the receptor is specifically directional, that of the ligand must be also, and movement of the carboxyl group to the face of the indole ring as shown in Fig. 2 is proposed as the only side chain movement able to confer hormonal activity. The ability of the side chain to move to the 'back' face (as depicted) is of no consequence to activity. Thus the contrasting steric locations of substituents in enantiomer pairs and the consequent effects on the ability of the side chain to move between recognition and modulation conformations is reflected in the general trends relating absolute configuration and hormonal activity. Side chain substituents in the *L*-configuration introduce intra-molecular steric hindrance to side chain movement, reducing hormonal activity and often imparting antagonistic properties. Generally the stereo-relationships imposed by the absolute *D*-configuration prohibit such intramolecular

effects. Methyl substituents on the side chain of auxins in the *D*-configuration may, however, reduce unfavourable electrostatic interactions with the electropositive region (iii) of the receptor site, resulting in increased affinity between ligand and receptor in the case of weak auxins⁹.

Data tabulated for the activities of all mono-, di- and tri-chlorophenoxyacetic acids¹¹ not only illustrate factor (1), but (3) also. All compounds displaying zero activity in the particular bioassays used by Leaper and Bishop possess either di-*ortho* substitution, or are unable, by face reversal (rotation about the bond linking the side chain to ring), to avoid placing a substituent in locations analogous to those which would be adopted in the site by substituents on C₇, C_{7a} or N₁ of IAA (di-*meta* phenoxyacetic acid derivatives, Fig 3); the reduced activities of 7-chloro-IAA¹³, N₁-methyl-IAA^{12,13}, 4-chloro- and 5-chloro-naphth-2-yloxyacetic acids¹⁷, relative to the unsubstituted parent acids, is also explained on the basis that the substitutions introduce novel interactions with the 'floor' of the receptor site, thereby reducing binding affinity.

Auxins of the arylcarboxylic acid type lack a spacing atom between the carboxyl group and ring structure, so that a conformational change of the type proposed for other auxins is not possible. Instead, arylcarboxylic auxins are proposed to show a molecular reorientation in conjunction with the conformational change of the receptor. As usual, recognition and binding occur with the oxygens of the carboxyl group astride the ring plane. Once in the site the entire molecule is envisaged to tilt through approximately 45° relative to the ring plane at recognition 'conformation', about an horizontal axis passing through ring

Fig. 4 2,6-dichlorobenzoic acid, with the alignment of the carboxyl group determining molecular orientation for the recognition 'conformation' (top). A modulation 'conformation' (below) equivalent to that adopted by IAA is assumed by partial rotation of the molecule about a horizontal axis passing through the centre of the ring.



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carbons 2 and 5; in this manner the molecule assumes an equivalent modulation conformation (Fig. 4). This theory would endorse the two salient features of Veldstra's conclusions²¹ concerning benzoic acid structure-activity relationships: a non-flat structure, with the carboxyl group oxygens astride the ring plane, is essential for binding; all other requirements with respect to substituents, including the di-*ortho* substitutions responsible for the non-flat structure, are suggestive that absence of intermolecular hindrances to binding in the receptor site is critical, rather than a requirement for the presence of specific unsubstituted locations on the ring for binding.

There is insufficient evidence to make further conclusions about structural requirements for activity in benzoic acid derivatives. The observation that *para* substituents on benzoic acid exceeding the size of fluorine are incompatible with activity²¹ would seem to require the proviso that consideration also be given to possible stabilisation of charged resonance forms of the ligand. Thus the inactivity of *para* chlorinated benzoic acids²¹ and data on their potency as antagonists of auxin action²²⁻²⁴ suggest that such derivatives are accepted into the site, but are unable to show a re-orientation in concert with the receptor conformation change; presumably steric interaction between the *para* chloro group and the 'floor' of the site prevents the required reorientation from occurring. The *modus operandi* of 4-amino-3,5,6-trichloropicolinic acid as a hormone will resemble that of the benzoic acids, yet despite the presence of a chloro substituent *para* to the carboxyl group, this compound can show hormonal activity exceeding that of IAA²⁵. It is thought that resonance stabilisation of charges could influence the activity of this compound in two relevant ways; first, molecular dimensions may be altered to render it sufficiently different from *para* chlorinated benzoic acids; and second, the positively charged amino group is able to assume a location in the binding site analogous with that of the pyrrole nitrogen of the indole ring of IAA, so that favourable interaction with the proposed electronegative region (iv) might reduce hindrance to the '*para*' chlorine substituent during the molecular re-orientation required for activity.

Figure 5 illustrates an empirical method of approximately summarising the maximum ligand dimensions permissible for binding under the canopy region of the site. The expected variation amongst receptor sites (tissue-specific and species-specific) will prevent adoption of a single critical dimension universally applicable to auxins for the distance between 'top' of the ligand carboxyl group (in the recognition conformation) and the lowest molecular extremity. The values chosen for Fig. 5, however, exclude such diverse inactive auxin analogues as *trans*-cinnamic acid²⁶, 2,6-dibromo-, 2,6-diiodo- and 2,6-dimethoxy-benzoic acids³, 9-anthracenecarboxylic acid²⁷ and indol-3-yl-carboxylic acid^{2,12}.

True antagonism to auxin action (as opposed to general metabolic inhibition) is considered, on this hypothesis, to be due to two general types of compounds. One category can be regarded as high affinity, low capacity auxins, as they occupy the site but are unable to show a conformational change or reorientation with the receptor. Examples of this type are L(-)- α -(2-methyl-4-chlorophenoxy)-propionic acid^{28,29}, 4-chloro- and 2,4-dichlorobenzoic acid^{23,24}, 2,6-dichlorophenoxyacetic acid³⁰, and auxins with isobutyric acid side chains³. A second class of antagonists have the properties of low affinity, high capacity auxins; although side chain flexibility leaves such molecules with the capacity to function as auxins, the nature of the remainder of the molecule reduces affinity for the receptor site and these antagonists compete with auxins for receptor regions (i) and (ii). Examples of this second type are 3,5-dichlorophenoxyacetic acid, and phenoxyacetic acid and its 2-halogen derivatives³. Åberg's discussion of antagonism of potent

auxin action by weak auxins would be viewed as this second form of antagonism. Gradations between these two classes of antagonists will occur.

The hormonal activities attributable to L(-)- α -(indol-3-yl)-propionic acid³¹⁻³³, α -(indol-3-yl)-isobutyric acid^{3,33}, and L(-)- α -(benzothien-3-yl)-propionic acid³⁴ remain unexplained by this theory. Molecular models of these compounds suggest their side chain movement to the modulation conformation is not possible, and the theory would predict antagonistic properties for them; indeed, this is the case for α -(indol-3-yl)-isobutyric acid³⁵ and L(-)- α -(benzothien-3-yl)-propionic acid³⁴ in some bioassays. High auxin activity and strong antagonism, however, are viewed as mutually exclusive features, and it is not without precedent to comment that these compounds deserve more rigorous investigation^{3,6}. All structures closely resemble that of the natural auxin IAA, and the contradictory results presently available for them may reflect metabolic conversions to active forms within some test tissues.

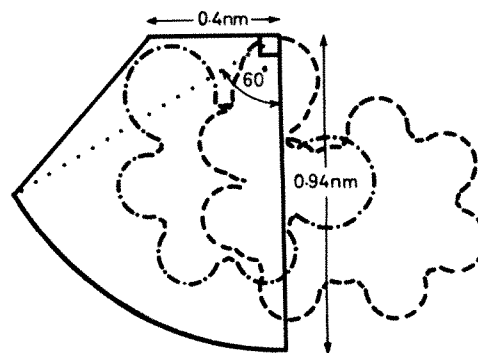


Fig. 5 An empirical method of approximately summarising the maximum ligand dimensions permissible for binding under the 'canopy' region of the receptor site (solid lines). Measurements are taken from the top of the carboxyl group when side chain (if present) and carboxyl group, in the recognition conformation, are aligned so as to coincide with the corresponding regions of IAA in its recognition conformation (IAA ----; 2,6-dichlorobenzoic acid - - - - -).

When this conformational change model for auxin action is discussed within the context of former theories of auxin structure-activity relationships, it is seen that it represents a rationalisation and extension of previous concepts. First, molecular significance can be attached to the five structural features characterising the many synthetic auxins known by 1938². A ring system provides the planarity to fit the cleft, an hydrophobic core for interactions with appropriate regions on the receptor site, and in the case of IAA, the molecular complexity to facilitate hydrogen bonding through the indole nitrogen region. Double bonds in the nucleus (or minimally, one double bond with the side chain attached to it³⁶) permit adoption of a recognition conformation with the side chain in the plane of the ring and a modulation conformation with the side chain perpendicular to the plane of the ring. A side chain arising from the ring nucleus provides the molecular flexibility required for simultaneous conformational changes with the receptor. A carboxyl group (or other acidic function) on the side chain is essential for auxin recognition by sites (i) and (ii); the stipulation that the carboxyl group be separated by one atom from the ring again relates to the need for molecular flexibility if hormonal activity is to be retained. The observation that a particular space relation between carboxyl group and ring was required for activity reflects the necessity for side chain conformational degeneracy so that the carboxyl group can move between two specific spatial locations defined by the extent of movement of the recognition

regions (i) and (ii) of the receptor as this shows its conformational change.

Furthermore, the proposed recognition conformation apparently coincides with the active auxin form suggested by Jönsson⁴, while the modulation conformation is equivalent to the active form proposed by Veldstra³. The premises of the two-point attachment theory³⁷ and three-point attachment and α -hydrogen theory³⁸ are encompassed by the hypothesis that side chain conformational changes are essential for auxin activity. The 5.5 Å (0.55 nm) charge separation theory³⁹ is a rigid hypothesis expressing the possible relevance of charged regions of auxins to receptor site binding; apart from the specific case of IAA, and the general requirement for an acid group on the ligand, it has little in common with this theory.

That auxin action entails more than a simple binding at a site has been intimated by others. Veldstra³ considered that auxins should possess "a capacity to cause a specific physiological response when fixed to the receptor (in dynamic equilibrium with free molecules!)". Similarly, discussing the influence of some alkyl side chain substituents on hormonal activity, Fredga and Åberg⁵ concluded that "the large substituent in some way hinders the side chain of the molecule from assuming the position leading to auxin activity". Unwittingly, both comments predict this hypothesis, that capacity to function as an auxin requires an ability to undergo a simultaneous conformational change or reorientation with the receptor.

An understanding of the biochemical significance of the proposed conformational change induced in the receptor by auxin binding must await characterisation of the site of auxin action. The nature of this conformational change theory of auxin structure-activity relationships is, however, compatible with current theories on the biochemical basis of auxin action, and not exclusively applicable to one only.

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Polymorphism of the major envelope glycoprotein (gp70) of murine C-type viruses: virion associated and differentiation antigens encoded by a multi-gene family

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Structural comparison of the major envelope glycoproteins (gp70) from 35 different murine type C viruses and free gp70 expressed at various anatomical sites in the mouse showed that the gp70s are polymorphic products of a large multi-gene family encoding viral and differentiation antigens. Different proviruses are expressed in cells following distinct pathways of differentiation. When the various gp70s are grouped according to primary structure they fall naturally into viral host range classes, confirming the suspicion that C-type viral tropism is largely determined by the nature of the gp70 product expressed.

POLYMORPHISM of certain viral proteins is well known and often forms the basis for the serological classification and subdivision of

antigenically related viruses¹⁻³. Viral polymorphism probably reflects the response of specific viral genes to selective pressure, and as for the influenza viruses, may influence the dynamics of the host-parasite relationship³. When, however, one considers the murine C-type viruses, the issue of polymorphism takes on a unique significance in that these agents may spend at least part of their life cycle as proviruses integrated into the host chromosomes where they may be inherited according to mendelian expectations⁴⁻⁶. One or more of the proviral genes may be expressed at certain sites of differentiation, often in the absence of complete virus production⁶⁻⁹. Insofar as expression of the provirus is regulated during cellular differentiation, its gene products may be considered among the differentiation antigens of the mouse¹⁰. There are two important questions regarding polymorphism of C-type viral proteins as structural components of intact virus and cellular differentiation markers. The first question concerns whether the gene products are 'virus-like' with extensive divergence or more

'host-like', where even the products of the most polymorphic loci are relatively conserved. Second, we need to know whether different proviruses are expressed at different sites of differentiation, resulting in the presence of multiple subtypes of related viral proteins within a single animal.

Of the various gene products of endogenous C-type viruses, the major envelope glycoprotein gp70 is well suited for a study of polymorphism. This glycoprotein has a role in the host range, interference and neutralisation properties of the virus and carries the type-, group- and interspecies determinants which form the basis for viral subclassification. But a classification based on the functional properties of viral gp70 does not allow comparison at the molecular level and cannot be used in the absence of infectious viruses. Therefore, a more rigorous approach to the study of

Tryptic peptides of gp69, gp70 and gp45 are identical

Initial experiments were performed to determine the value of ^{125}I tryptic peptide analysis as a tool for structural comparison of related gp70s. One issue we needed to resolve concerned the possible presence of more than one gp70 in a given virus preparation, as was suggested by the early finding that Rauscher gp70 could be resolved into discrete bands with molecular sizes of 69,000 and 71,000 (refs 11, 12). If gp69 and gp71 were different gene products this would obviously complicate a structural study of polymorphism. In addition, we studied a protein with molecular weight of 45,000 which is precipitable by anti-gp70 antisera and is found in certain virus preparations. In Fig. 1 the tryptic peptide

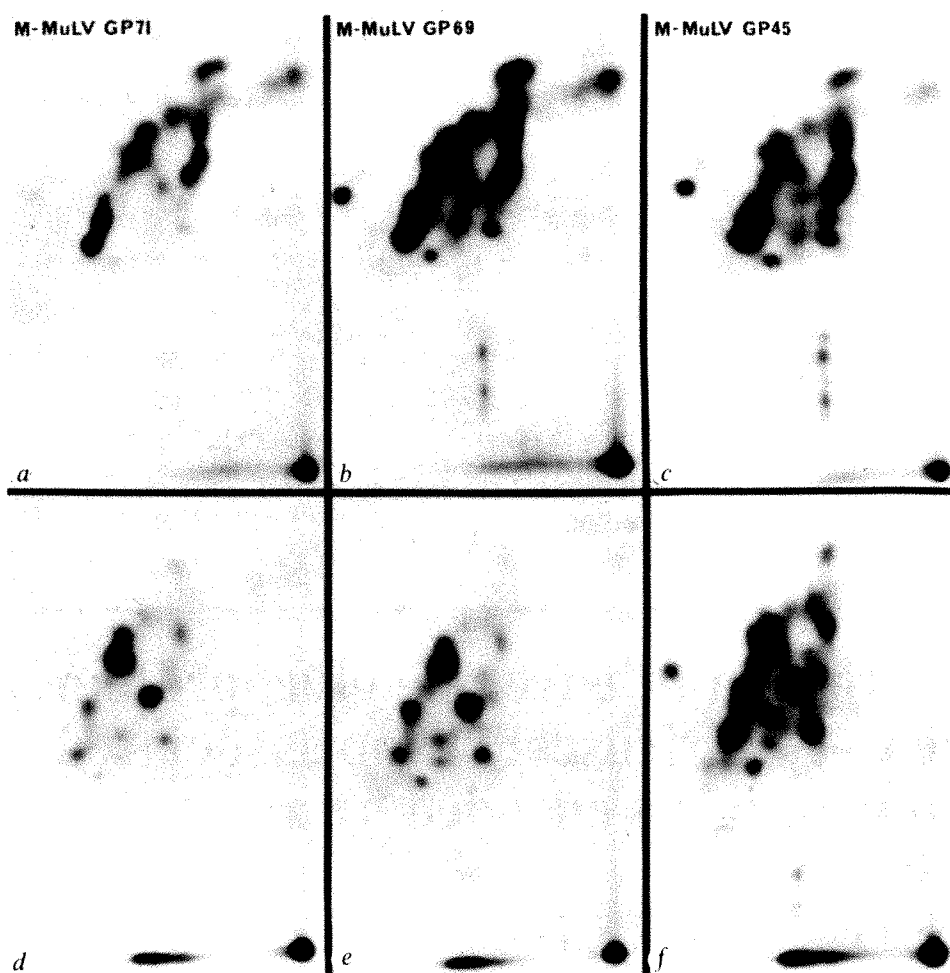
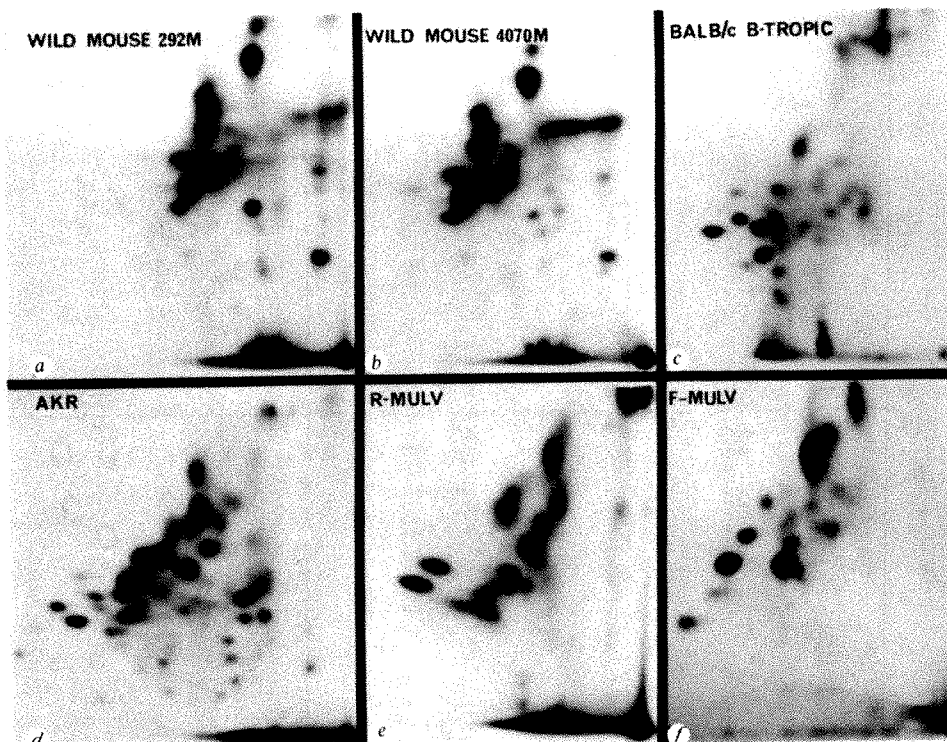


Fig. 1 Comparison of the tyrosine-containing tryptic peptides of Moloney virus gp71, gp69 and gp45. Moloney virus gp69/71 was purified by phosphocellulose chromatography and was a gift from Dr S. Kennel of Oak Ridge Laboratories. The protein (10 μg) was radiolabeled with ^{125}I (Amersham, specific activity about 15 $\text{mCi } \mu\text{g}^{-1}$) to high specific activity by the chloramine T method¹³ in the presence of either 0.5% NP40 in 0.5 M phosphate buffer (a-c) or 1% SDS in 8 M urea (d-f). Gp71, gp69 and gp45 were then separated by SDS-PAGE¹⁴, the gels were sliced and the slices containing the radioactive proteins were pooled and placed in 10% acetic acid. The slices were washed extensively with 10% methanol to remove SDS, dried with a heat lamp, and placed in a solution of 50 $\mu\text{g ml}^{-1}$ trypsin (Worthington, 27 IU mg^{-1}) in 0.05 M NH_4HCO_3 buffer (pH 8.0). The slices were incubated overnight at 37 °C, after which the solution was removed and lyophilized. The tryptic digests were then analysed on two-dimensional peptide maps¹⁵. The samples were dissolved in 20 μl buffer I (acetic acid-formic acid-water, 15:5:80) and 2–5 μl was spotted on to 10 cm $\times$ 10 cm cellulose-coated TLC plates (EM Laboratories). The samples were then electrophoresed at 1 kV for approx. 30 min, dried and chromatographed in a second plane using buffer II (butanol-pyridine-acetic acid-water, 32:5:25:5:20). The plates were then dried and analysed by autoradiography using Kodak RP-X-ray film. a-c, gp71, 69 and 45, respectively, radiolabeled in NP40; d-f, GP71, 69 and 45 radiolabeled in the presence of 1% SDS and 8 M urea.

polymorphism requires the use of techniques allowing direct comparison of primary structure. We report our comparison of the primary structure of 27 different virion-associated gp70 molecules and gp70 isolated from the sera and seminal fluid of nine different strains of mice. Based on the tyrosine-containing tryptic peptides of these gp70s, the C-type viruses of murine origin can be divided into several groups with varied degrees of relatedness. Polymorphism exists within each of these groups. Compared to viruses recently isolated from inbred or from feral mouse populations, the gp70s of the laboratory-derived murine viruses vary extensively in structure. The structure of the gp70 found free in the serum of all mice tested is relatively conserved and seems to be the product of the same or similar xenotropic virus gene regardless of how many other proviruses the mouse may harbour. The provirus coding for the serum protein is distinct from that coding for the genital tract gp70 and thus unique proviruses are expressed at different sites of differentiation.

analyses of gp71, gp69 and gp45 present in a preparation of purified Moloney virus gp70 are shown. Figure 1a-c shows that the tyrosine-containing peptides of these three proteins are identical. Another question answered in this experiment is the extent to which the carbohydrate portion of the molecule influences the resultant tryptic map. It is estimated that 32% of the mass of gp70 is carbohydrate, whereas carbohydrate is only 6–9% of gp45¹⁶. Because the tryptic maps of gp69, gp71 and gp45 are identical, it is apparent that the mass of carbohydrate does not greatly influence our analyses and this is because the glycopeptides do not migrate in the apolar solvent used here for chromatography (our unpublished results). Another methodological question concerned the extent to which conformation of the molecule influenced radioiodination and the subsequent peptide map. Figure 1a-c shows the tryptic peptides of the proteins iodinated in the presence of NP40 (0.5%) and 0.05 M sodium phosphate buffer; in Fig. 1d-e these proteins are radioiodinated in the presence of 1% SDS and 8 M urea.

Fig. 2 Comparison of the tyrosine-containing tryptic peptides of gp70 isolated from several endogenous and exogenous ecotropic (mouse-tropic) viruses. *a*, Wild mouse 292M; *b*, wild mouse 4070M; *c*, BALB/c B-tropic; *d*, AKR; *e*, R-MuLV; *f*, F-MuLV. Rauscher virus, produced in chronically infected JLSV-9 cells, was obtained from Dr Jack Gruber of the Resources branch of the NCI. SC-1 cells producing AKR virus (AKV-1) were obtained from Drs W. Rowe and J. Hartley and virus pools were prepared from the third and fourth subculture in our laboratory. Friend virus¹⁷ was a gift of Dr W. Parks. B-tropic virus (no. 138) was isolated from a 3-methylcholanthrene-induced tumour of a BALB/c mouse. The no. 138 virus is completely restricted in FV-1ⁿ cells even at high multiplicity of infection. Pools were prepared from the third to fifth tissue culture passage for use in this study. The origin and clonal purification of wild mouse ecotropic viruses (1504M, 292M, 4070M, 1740M) have been described¹⁸. Enriched fractions of gp70 were obtained from virus isolates by immunoaffinity chromatography as described¹⁹ with several modifications. Monospecific goat antisera to Rauscher virus gp70 (G230) was used as the source of antibody. Proteins were eluted from the affinity column with a 0–2 M TCA gradient at neutral pH as suggested²⁰. The majority of non-specific protein came off the column from 0 to 0.75 M TCA. Gp70 was eluted from the column at 1–1.5 M TCA. After dialysis against phosphate-buffered saline, gp70 from all isolates retained antigenicity and remained stable for several months at –20 °C. After dialysis, the gp70 fractions were radioiodinated as in Fig. 1 and direct precipitates were performed with G230 antiserum. The immune precipitates were then electrophoresed on SDS-PAGE and treated for peptide mapping as described in Fig. 1.



Changes in the intensity of certain spots have occurred, but the total pattern is the same. This suggests that conformation of the gp70 molecule may influence the extent of iodination of certain peptides but all peptides are labelled even under relatively mild conditions.

The virion-associated gp70s can be divided into groups

Based on tryptic peptide analyses of 27 virion-associated gp70s, the following conclusions are drawn: (1) The gp70s of the murine viruses which both grow in and infect mouse cells (ecotropic viruses) can be divided into several divergent groups. This point is illustrated in Fig. 2, in which the tryptic peptides of 6 endogenous and exogenous ecotropic viruses are shown. Overall, the gp70s of the various wild-type ecotropic (*a–d*) viruses are more related to each other than to the FMR (*b, f*, Fig. 1*a*) viruses. The gp70s of the ecotropic viruses of wild mice (Fig. 2*a, b*) form a closely related group, distinct in structure from the prototype AKR viruses (*d*). The gp70s of FMR viruses, Friend, (Fig. 2*f*), Rauscher (Fig. 2*e*) and Moloney (Fig. 1) are divergent from each other and the other ecotropic viruses, possibly as the result of a recombinant origin. These data indicate that extensive diversity exists from one group to another in spite of the fact that ecotropic gp70s share the common property of facilitating entry of their respective viruses into mouse cells, but not cells of other species. (2) The gp70s of the endogenous viruses which are produced by mouse cells, but can only infect cells of other species (xenotropic viruses), form two groups, one of which is closely related to the gp70s of viruses which can infect both mouse cells and cells of other species (amphotropic). In Fig. 3, the tryptic peptides of several xenotropic virus gp70s and an amphotropic virus gp70 are compared. BALB/c, AKR and NIH xenotropic gp70s are closely related to one another and to the amphotropic gp70, but are distinct from the NZB xenotropic virus gp70. We interpret the differences within the large group of xenotropic gp70s (as well as the gp70s of C₃H and NZW, not shown) as polymorphism within a group. The gp70 of the NZB xenotropic virus is divergent to the point that we place it along with (NZB × NZW) F₁ (not shown) into a separate group of xeno-

tropic virus. These findings are in general agreement with serological studies of others²³. (3) The gp70s of amphotropic wild mouse viruses form a single highly conserved group closely related to one of the xenotropic groups (Fig. 3). The structure of the amphotropic gp70s will be completely described in a separate report (Bryant *et al.*, in preparation).

To determine whether the cell in which the viruses were propagated influenced our results, we compared the structure of the gp70s of three different amphotropic viruses, each of which had been grown in mouse, rabbit and mink cells. The gp70s were identical irrespective of in which cells the viruses were grown. This suggests that host cell modification of gp70 is not a significant variable in our studies.

The serum gp70 of laboratory and wild mice is homologous to the NZB xenotropic virus gp70

Large amounts of gp70 are present in the serum of many strains of mice in the absence of intact viruses⁷. To determine which provirus(es) encode serum gp70 we compared the tryptic peptides of gp70 isolated from the serum of several strains of mice. In Fig. 4, the tryptic peptides of virion-associated NZB gp70 (*a*) and 5 gp70s isolated from sera of different murine strains (*b–f*) are shown. The data indicate that the serum gp70s are members of a highly conserved group, all of which are closely related to the NZB xenotropic virus gp70. Additionally, NZW and two wild mouse serum gp70s had the same structure (data not shown). Thus, the serum gp70 of all mice we have tested is probably a partial expression of a single provirus, which is expressed as complete virus only in the NZB and (NZB × NZW)F₁ mice. In so far as that part of the provirus coding for serum gp70 is concerned, there has been little divergence and it appears to behave like a moderately polymorphic murine gene. These structural studies support the data of Stephenson and Aaronson in which it was shown that serologically the serum gp70 resembled that of the NZB xenotropic virus²³.

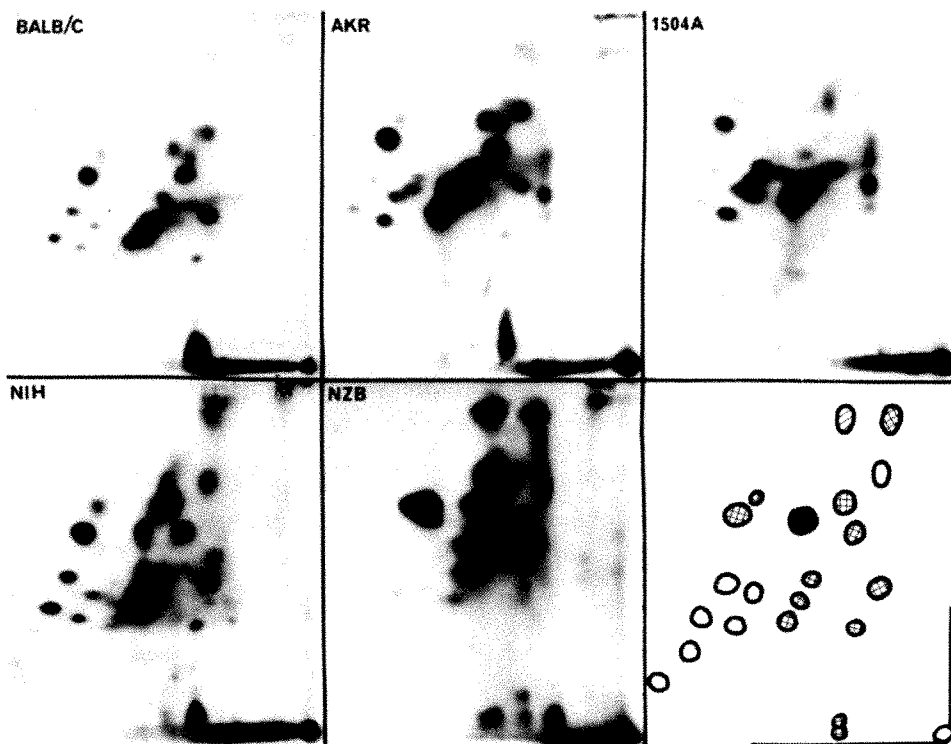


Fig. 3 Comparison of the tyrosine-containing tryptic peptides of several xenotropic virus gp70s and amphotropic virus gp70. *a*, BALB/c; *b*, AKR; *c*, 1504A; *d*, NIH; *e*, NZB. Xenotropic virus isolates were obtained from spleen lymphocytes or from lung fibroblasts of normal AKR, NZB, (NZB x NZW) F_1 , C $_3$ H and NZW mice by co-cultivation with rabbit endothelial cells. The rabbit endothelial cells were derived from the pulmonary vein or from the vena cava of a New Zealand rabbit. For isolation of xenotropic viruses, endothelial cells were used from the fifth to twentieth tissue culture passages. Virus replication was monitored by RNA-dependent DNA polymerase assays. All xenotropic virus isolates were characterised by their ability to rescue the murine sarcoma virus genome from MSV-transformed non-producer rat cells (1255-B7), by radioimmune assays for p30, and by the lack of replication in secondary mouse fibroblasts of FV-1 ^{B} or FV-1 ^{b} strains. In all cases, xenotropic virus recovered from either spleen or lung yielded the same result in our analyses. The cloned amphotropic isolate (1504A) was prepared as described¹⁸. Amphotropic viruses have the combined host range characteristics of both mouse-tropic and xenotropic

Distinct proviruses are expressed at different sites of differentiation

To study whether different proviruses are expressed at different sites of differentiation we compared the structure of gp70s isolated from different anatomical sites in the same mouse. Figure 5 shows the tryptic peptides of the gp70 isolated from the serum and seminal fluid of 129 mice. Clearly the two proteins are distinct. The serum protein resembles gp70 of the NZB xenotropic virus whereas the seminal fluid protein is like the FMR group in

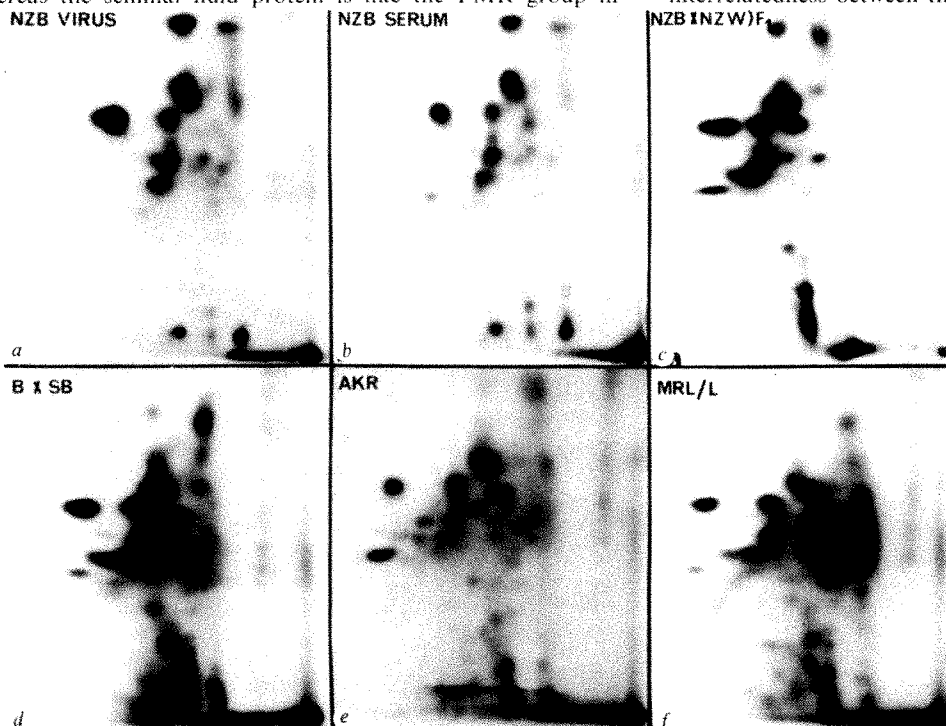
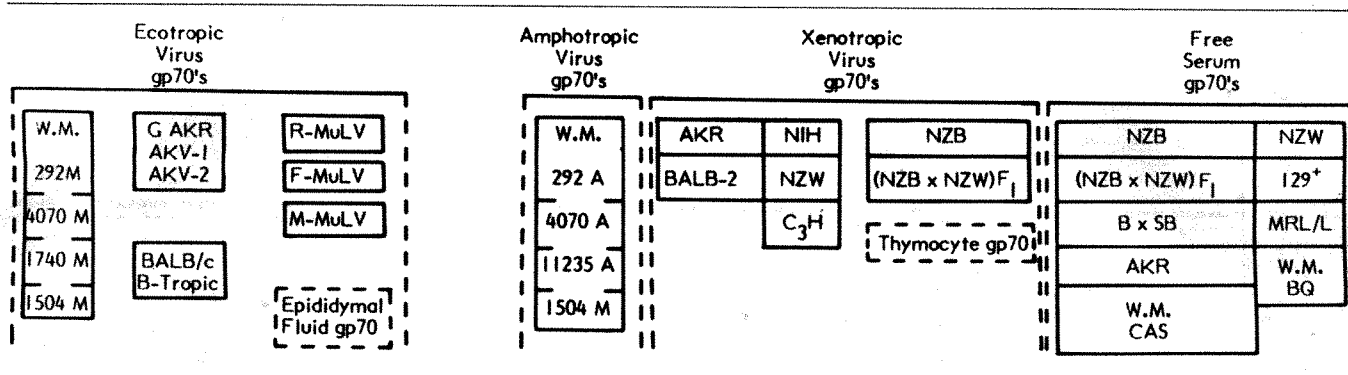


Fig. 4 Comparison of the tryptic peptides of NZB virus gp70 with the tryptic peptides of gp70s isolated from the serum of several strains of mice. *a*, NZB virus; *b*, NZB serum; *c*, (NZB x NZW) F_1 ; *d*, B x SB; *e*, AKR; *f*, MRL/L. Serum gp70s were isolated by immunoaffinity chromatography and treated for peptide map analysis essentially as described in Fig. 2. EDTA (10 mM) and EGTA (10 mM) were added to the sera prior to passage over the immunoaffinity column to prevent complement binding and reduce proteolysis. B x SB is a new recombinant inbred strain derived from a single (C57 B1/6 x SB/Le) F_1 cross with selection for the mutant satin (*sa*) and beige (*ba*) genes developed by Dr Edwin D. Murphy of Jackson Laboratories. MRL/L is a new recombinant inbred strain obtained from the twelfth generation of an inbred stock derived from strains LG, AKR, C $_3$ H and C57 B1/6, also developed by Dr Murphy. Analysis of the gp70 from the sera of wild mice from two separate populations (Lake Casitas and Bouquet Canyon²⁴) had similar homology to the NZB xenotropic virus gp70 (not shown).

viruses, but different interference and neutralisation specificities and are negative in the XC test^{18,21,22}. BALB/c (no. 435) and NIH Swiss (no. 436) xenotropic viruses were a gift of Drs Hino, Stephenson and Aaronson of NIH and their origin and purification have been described²³. Gp70s were isolated and analysed as described in Fig. 2. *f*, Composite of the major spots of the xenotropic and amphotropic virus gp70s, indicating the degree of interrelatedness between the molecules. Crosshatched areas, common to all; ○, common to amphotropic gp70 and to *a*, *b* and *d* (as well as C $_3$ H and NZW, not shown); ●, common to NIH and NZB; ○, common to NZB (and NZB x NZW F_1 , not shown).

Formulation for members of the multi-gene family

Table 1 summarises all the data presented above. All the virion, serum and tissue proteins on the diagram are immunologically related and thus can be considered expressions of a multigene family²⁶. Groups are represented as joined boxes which are spatially placed to indicate our estimation of the extent of interrelatedness between the groups. Based on our preliminary

Table 1 Summary diagram of the structural relationship between all virion- and non-virion-associated gp70s analysed

Joined boxes represent closely related groups and the spatial arrangement of these groups is intended to indicate intergroup relatedness. The gp70s found free in the epididymal fluid and on the surface of thymocytes are surrounded by broken lines and are placed according to their structural relatedness to virion-associated gp70s. W.M., wild mouse; C.A.S., Casitas trapping area; B.Q., Bouquet Canyon trapping area.

structural studies, the thymocyte-associated gp70 is placed in the xenotropic group. We are now doing the N-terminal sequence of a number of these gp70s to determine precisely the inter- and intra-group divergence.

Discussion

We show here that the various virion-associated and free serum gp70 molecules can be divided into groups. The extensive inter-group divergence of the FMR ecotropic murine viruses may be due to their unique origins but may also reflect recombinations during their laboratory history. By contrast, minimal divergence is seen among the members of groups of endogenous viruses very recently isolated from inbred or wild mice. Thus, given the opportunity for recombination and/or replication outside the host, oncornaviruses may diverge extensively. But, under mendelian restrictions members of each group remain relatively conserved, suggesting that selection operates at the level of the integrated provirus.

It is clear that the endogenous free or virion-associated gp70s are part of a multigene family of immunologically and structurally related proteins. An important question is: how many members does the family contain? We believe that the family may be extensive, but at present we can only give a lower limit for expression of at least five members within a given mouse. For example, in an AKR mouse there are two AKV genes, one AKR xenotropic virus gene, one gene for the serum gp70, and a gene for one of the proteins secreted into the male genital tract²⁵. This accounting does not include at least five additional gp70s expressed at other sites of differentiation¹⁰, as we have not yet

isolated these to determine if they differ from the known gp70s.

In the case of the endogenous AKR provirus, it has already been shown that two presumably identical genes (AKV-1 and AKV-2) have different genetic loci²⁷. But, in spite of the different loci, our recent studies with Hartley and Rowe have shown that the gp70s of viruses derived from AKV-1 and AKV-2 are identical. Thus with respect to the xenotropic proviruses, the question is whether they are also non-alleles or simply very polymorphic products of a single locus. Our results show that there are at least two loci for xenotropic proviruses in that in most mice a virus can be recovered from many tissues which has a gp70 distinct from that present in the serum of the same mouse. The provirus encoding the serum protein can be considered xenotropic in that in the exceptional strain (that is, NZB) when this gp70 becomes part of a virion, it confers xenotropic host range. In so far as the genes for the complete xenotropic viruses of different strains are concerned, our data do not allow us to distinguish between non-alleles against extensive polymorphism at a locus.

If we accept the assumption that the various endogenous proviruses are non-alleles, then a very interesting question concerning regulation of their expression emerges. Studies in our laboratory and Boyse's have already shown that in the mouse, gp70 is expressed only in cells following certain pathways of differentiation^{4,9}. Now the issue becomes whether at each site of differentiation the gp70 is the product of a different provirus. Although we cannot yet be precise about the answer, there are already some observations which suggest that at each site of differentiation one or more different proviruses are expressed. For example, as mentioned above, within a strain the serum and one of the genital tract gp70s have different tryptic peptides. In terms of neoplasia if the proviruses expressed at different sites of differentiation are not genotypically equivalent then one can begin to understand how expression of the viral genome at one site can have oncogenic consequences distinct from another. Regardless of to what extent we are dealing with polymorphism against multiple genes, it is already clear that the study of endogenously expressed gp70 can give important insights into the extent and perhaps mechanisms of divergence of a well-defined murine glycoprotein. The uniqueness of the situation, of course, stems from the fact that because of the extrachromosomal potential of the provirus, it is often possible to have both the gene and gene product in hand.

We thank Drs S. Hino, J. Stephenson, S. Aaronson, M. Gardner, W. Parks, W. P. Rowe and J. Hartley for certain viruses used in this study and for discussions. We thank Dr F. Dixon for mouse sera, and acknowledge the technical assistance of Ms J. Hampton and A. Chen. This research was supported by a grant from the US NCI.

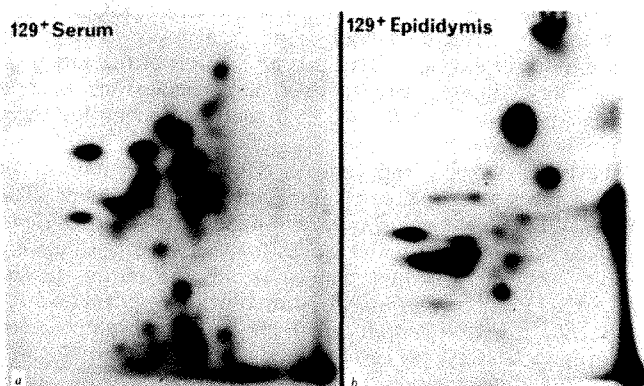
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Fig. 5 Comparison of the tyrosine-containing tryptic peptides of gp70s found free in the serum (a) and epididymal fluid (b) of mice. Gp70 was isolated from the serum of 129 G₁X⁺ mice as in Fig. 5 and analysed as in Fig. 2. The epididymal fluid gp70 was radioiodinated and immune precipitated from the epididymal secretion of male 129 G₁X⁺ mice as previously described²⁵.



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letters to nature

Galactic distribution of X-ray burst sources

By applying the following working definition for X-ray bursts¹—rise times of less than a few seconds, durations of seconds to minutes, and recurrence in some 'regular' pattern—18 burst sources have been observed to date (refs 2–16) of which one (observed by SAS-3) has not yet been reported. Their positions are shown in Fig. 1. If single 'burst' events which meet the criteria of rise time and duration but not recurrence are accepted, seven burst sources can be added^{16–19}, of which two (observed by OSO-8) have not yet been reported. These seven are also indicated in Fig. 1.

Many other 'burst' events have been reported in the literature^{20–23} but are omitted here, since they meet neither the criteria of rise time nor recurrence. It is very likely, however, that some of the many events observed by the Los Alamos group^{20–22} were X-ray bursts of the kind discussed here. Evans (private communication) believes that perhaps five or six burst sources have been found by them. Since sufficient knowledge about the rise times and the recurrence pattern of these events is lacking, they are not included in Fig. 1.

The burst sources are spread along the galactic equator and cluster near low galactic longitudes ($|l| < 40^\circ$). Lewin¹⁸ concluded, on the basis of the positions for 15 X-ray burst sources, that their distribution is different from that of the observed globular clusters²⁴. On the basis of an even smaller sample of some of the SAS-3 results, Lewin¹ also concluded that the

distribution of burst sources is unlike that of the previously known (catalogued) galactic X-ray sources²⁵, but pointed out that the burst sources could well be a subclass of the galactic X-ray sources.

The SAS-3 X-ray observatory and the Goddard X-ray Spectroscopy Experiment on OSO-8 have discovered 21 of the 25 burst sources shown in Fig. 1. Here we examine the distribution of burst sources and evaluate (with improved statistics) the effect of uneven sky exposure on the observed distribution, using data from these two observatories. In our analysis we include sources from which single 'burst' events have been observed; none of our conclusions would change if we ignored them.

From the SAS-3 observatory, we present only data from the rotating modulation collimator detectors²⁶ (RMC), which have $\sim 12^\circ \times 12^\circ$ (FWHM) fields of view. The RMC systems view the sky in the direction of the spin axis ($+z$ axis). Their view direction therefore does not change rapidly, and the sky exposure can be easily calculated. We have thus ignored several sources from which bursts were detected in the SAS-3 y -axis detector systems only^{7,8,15}.

The OSO-8 burst observations were made with three independent detectors viewing the directions of the $+z$ axis (spin axis) and that of the $-z$ axis. The detector that views the sky in the $+z$ direction has a circular field of view of $\sim 5^\circ$ FWHM. Two detectors view the sky in the direction of the $-z$ axis; one has a circular field of view of $\sim 3^\circ$ FWHM (centred on the $-z$ axis) and one has a circular field of view of $\sim 5^\circ$ FWHM (this one is offset from the $-z$ axis by 5°). These OSO-8 detectors have been described in more detail by Serlemitsos *et al.*²⁷. For the present purpose, the two $-z$ axis detectors can be considered as one detector system with a circular field of view of radius $\sim 10^\circ$ centred on the $-z$ axis. This is certainly valid for observations of bursts that last longer than ~ 20 s, since the spin period of OSO-8 is ~ 10 s. For shorter bursts, the equivalent field of view decreases by a factor of up to 2.

The sensitivity of each detector for detecting bursts depends on the detector area, the background level, the field of view and the exposure time. For the SAS-3 RMC and the OSO-8 $5^\circ - z$ axis detectors, the sensitivity is also adversely affected by modulations of strong sources due to rotation of the satellites. We do not attempt here to draw any conclusions from a comparison of the number of burst sources seen by the three different detectors.

The calculated sky exposure (in days) and the number of burst sources observed are shown in Tables 1 and 2. The results were calculated using 'clean' data only. As a result of Earth occultation, the actual values for the sky exposure were $\sim 30\%$ less (mean value) than those shown in the tables. The SAS-3 results are primarily from 'quick look' data ($\sim 50\%$ of the total data collected) during the first 19 months of observations (up to January, 1977). The OSO-8 results are from the first 16 months of observations (up to October 1976); for the last 4

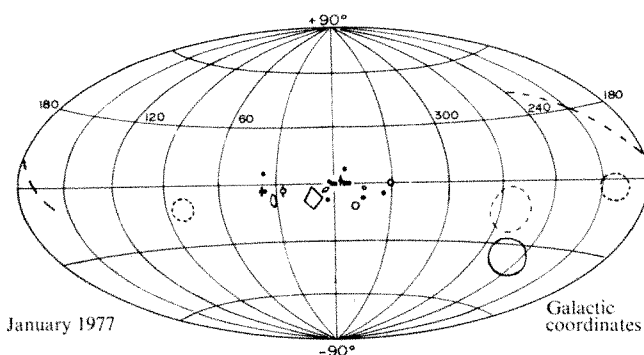


Fig. 1 Sky map (galactic coordinates) of 18 X-ray burst sources which meet the three burst criteria (see text). Seven additional sources are shown for which the recurrence criterion of the bursts has not (yet?) been met. They are indicated with dashed lines (four sources) or with vertical bars (three sources). Open error boxes have areas ≥ 3 deg². The dashed line near the galactic anticentre represents an error box of $\sim 120^\circ \times 2^\circ$. The figure contains only burst sources discovered before 6 January, 1977. For more detailed information see ref. 18.

Table 1 Galactic latitude distribution of observed X-ray burst sources

$ b'' $	SAS-3 RMC data only		OSO-8 data		Arp catalogue Globular clusters
	Exposure* (d)	Burst sources observed	Exposure* (d)	Burst sources observed	
$< 20^\circ$	108	9	71	13	~ 75
$> 20^\circ$	207	1	34	0	~ 45

*Not corrected for Earth occultation

months, only 'quick look' data ($\sim 10\%$ of the total) are available. For OSO-8, the sky exposure at any absolute value of the galactic latitude is obviously the same for the $+z$ and the $-z$ detectors. We have therefore combined the data of the OSO-8 $+z$ and $-z$ detectors in Table 1 (but not in Table 2).

The numbers of burst sources listed in Table 1 observed by SAS-3 (RMC) and OSO-8 at low galactic latitudes ($|b''| < 20^\circ$) are nine and thirteen respectively. (These numbers are lower limits because of possible source confusion. We estimate that perhaps two or three more sources were detected in the region of high burst source density within $\sim 10^\circ$ of the galactic centre.) Three of the thirteen sources observed by OSO-8 were also observed by SAS-3 (RMC). Therefore, nineteen different burst sources are observed in the region $|b''| < 20^\circ$. One burst source was observed at galactic latitude $|b''| > 20^\circ$; this source, observed by SAS-3, has not been reported previously. The sky exposure for SAS-3 in the region $|b''| > 20^\circ$ was about twice that in the region $|b''| < 20^\circ$; for OSO-8 the exposure at low galactic latitude was about twice that at high galactic latitude.

The combined results of SAS-3 and OSO-8 illustrate unambiguously that the galactic latitude distribution of X-ray burst sources is very different from that of the known globular clusters²⁴ (see last column Table 1).

The galactic longitude distribution of X-ray sources for which $|b''| < 20^\circ$ is shown in Table 2. Again, the numbers of observed sources given for the region $|l''| < 60^\circ$ are lower limits (see above). In regions of low galactic longitude ($|l''| < 60^\circ$) the OSO-8 $-z$ and $+z$ detectors observed nine and six

We conclude from the combined OSO-8 and SAS-3 results of Table 2 that the galactic longitude distribution of X-ray burst sources is different from that of the low galactic latitude X-ray sources listed in the 3U catalogue²⁵ (see Table 2). It is quite possible, however, that the burst sources are a subclass of the low galactic latitude X-ray sources. Five of the twenty-five burst sources shown in Fig. 1 and possibly many more of them (refs 1, 9, 16, 18, 19) are associated with sources of steady X-ray emission.

It is interesting to note that the galactic longitude distribution for known globular clusters (in the region $|b''| < 20^\circ$) is similar to that of burst sources (see last column, Table 2). It has been proposed (see discussion in ref. 1) that perhaps the majority of burst sources are located only in those globular clusters near the galactic plane, but as pointed out earlier¹ this is very unlikely. There are at present sixteen X-ray burst sources whose positional uncertainties exclude known globular clusters; two burst sources are located in globular clusters (one in NGC6624 and the rapid burster), and seven additional burst sources could be located in globular clusters (it is very unlikely that all seven are). Therefore, of the ~ 65 known globular clusters (ref. 24) in the region $|b''| < 15^\circ$, two or at most eight are known to produce bursts. Thus, for every known globular cluster that is known to produce bursts there are ~ 8 – 30 that are not known to produce bursts. If the 16 burst sources whose positions exclude known globular clusters were located in obscured globular clusters, and if the occurrence of a burst source is as probable in obscured clusters as in the known ones, then there would have to be an additional 130–480 obscured globular clusters within 15° of the galactic plane. Their distribution would be very different from the spherical distribution of the known globular clusters, and they would constitute, in effect, a distinct disk population of globular clusters. To avoid this, one might assume that an intense concentration of matter in the immediate vicinity of many globular clusters is responsible for the obscuration of these clusters and that the presence of this obscuring matter somehow increases the probability of the occurrence of burst sources. We believe it is more likely that the majority of X-ray burst sources are not

Table 2 Galactic longitude distribution of observed X-ray burst sources for $|b''| < 20^\circ$

$ l'' $	SAS-3 (RMC)		OSO-8 ($-z$)		OSO-8 ($+z$)		3U Catalogue	Arp catalogue globular clusters
	Exposure* (d)	Burst sources observed	Exposure* (d)	Burst sources observed	Exposure* (d)	Burst sources observed		
0° – 60°	43	8	14	9	25	6	53	~ 71
60° – 120°	32	1†	32	1	32	0	29	5
120° – 180°	32	0	25	1	14	0	15	2

*Not corrected for Earth occultation.

†The source at $l'' \sim 250^\circ$ (see Fig. 1) is located at $|b''| > 20^\circ$ and is therefore not included.

burst sources, respectively. Four of these sources were observed by both detectors. Therefore, eleven different burst sources were detected by OSO-8, of which three were also observed by SAS-3. Thus, a total of sixteen different burst sources were observed by the two observatories in the region of low galactic longitude ($|l''| < 60^\circ$). One burst source was observed by SAS-3 near galactic longitude $l'' \sim 114^\circ$ (refs 17, 18) and one was observed by OSO-8 (unpublished) near $l'' \sim 79^\circ$. OSO-8 observed one burst source (unpublished) at high galactic longitude $|l''| = 120^\circ$ – 180° . (One other burst source was observed at high galactic longitude with one of the SAS-3 y -axis detector systems (dashed line in Fig. 1, refs 1, 18). The Los Alamos group may have detected three additional burst sources (refs 20–22) in this region. However, as mentioned earlier, the 'burst' character of these events is not yet established. The source at $l'' \sim 250^\circ$ (see Fig. 1) is located at $|b''| > 20^\circ$ and is therefore not included in Table 2.)

located in globular clusters of the kind that we know. This conclusion is supported by the direct observational evidence of Liller, who systematically searched for globular clusters in the vicinity of six burst sources and found only one²⁸. In five cases no globular clusters were apparent on his infrared plates (ref. 29). Liller believes that, in the case of MXB1728-34 for example, he would have detected a globular cluster out to a distance of ~ 19 kpc (ref. 29).

It has also been suggested (discussion in ref. 1; ref. 30 and T. Gold, unpublished) that the bursts are perhaps produced by remnants of disrupted globular clusters, specifically super-massive black holes. Such objects could perhaps accrete a sufficient amount of gas in the galactic plane (but not away from the plane) to produce bursts. Again, this would imply the existence of a new class of unknown objects and is at present merely an *ad hoc* method of relating the burst sources to globular clusters.

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First mass spectrometric measurements of positive ions in the stratosphere

ROCKET and balloon-borne electrostatic probe measurements have shown that positive and negative ions are present in the stratosphere reaching number densities between 100 and 10,000 cm^{-3} (refs 1-5). The observed densities indicate that galactic cosmic radiation is the major ionisation source and mutual recombination is the dominant ion loss process. No experimental information exists, however, on the nature of stratospheric ions. Here we communicate the first mass spectrometric measurements of positive ions in the stratosphere.

Three experiments were carried out at high latitudes (experiment F 33, 31 May 1972, 06.53 UT, latitude 69°N; experiment F 42, 10 June 1975, 13.19 UT, latitude 69°N; experiment S 18-1, 21 February 1976, 19.42 UT, latitude 68°N) using rocket-borne cryogenically pumped quadrupole mass filters. Mass spectra of positive ions at stratospheric heights were obtained between 24 km and 60 km during the descent portions of the rocket flights.

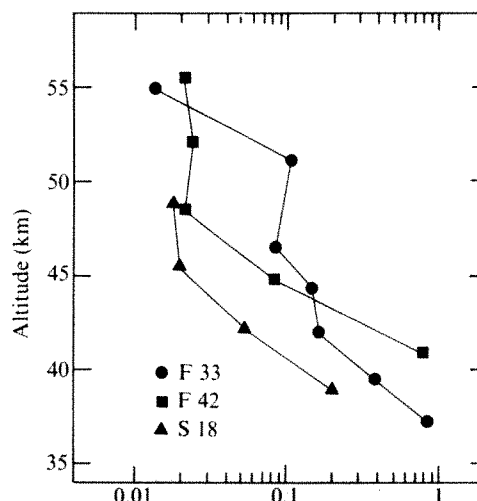
The mass numbers of the most abundant ions detected are listed in Table 1 along with tentative identifications. The uncertainty of ± 2 in atomic mass numbers is due to a relatively low mass resolution used in favour of high sensitivity. Above about 40 km the masses 19 ± 2 , 37 ± 2 , 55 ± 2 and 73 ± 2 are most abundant which are identified as proton hydrates (PH)

Table 1 Observed mass numbers and tentative identifications of ions

Mass (AMU)	Tentative identification of ions
19 ± 2	H_3O^+
29 ± 2	$\text{H}^+\text{CH}_2\text{O}$
37 ± 2	$\text{H}^+(\text{H}_2\text{O})_2$
42 ± 2	$\text{C}_2\text{H}_2\text{O}^+$
55 ± 2	$\text{H}^+(\text{H}_2\text{O})_3$
60 ± 2	$\text{H}^+(\text{CH}_2\text{O})_2$; $\text{C}_2\text{H}_2\text{O}^+\text{H}_2\text{O}$
80 ± 2	$\text{H}^+(\text{CH}_2\text{O})_2\text{H}_2\text{O}$; $\text{C}_2\text{H}_2\text{O}^+(\text{H}_2\text{O})_2$

$\text{H}^+(\text{H}_2\text{O})_n$ with n ranging from 1 to 4. It should be noted that PH were also found above 55 km in the mesosphere by these experiments. Here the mass filters were additionally operated in a second mode using higher mass resolution. Therefore, the identification of the above masses as PH seems to be quite safe. Below about 40 km the PH rapidly disappear and ions having masses 29 ± 2 , 42 ± 2 , 60 ± 2 and 80 ± 2 become most abundant. The change in the ion population occurs within only a few kilometers as demonstrated by Fig. 1 showing the fractional abundance of non-proton hydrates (NPH). The NPH probably result from PH reacting with one or more stratospheric trace gases by switching or proton transfer reactions. The unknown reaction partner should have either a higher permanent dipole moment or a higher proton affinity than H_2O or both and its number density should steeply decrease with height around 40 km. An inspection of molecules which satisfy condition one and which after adding a proton fall into the mass interval 29 ± 2 AMU corresponding to the observed ion of smallest mass yields the following candidates: CH_2O , HCN , CH_3N and CH_3NH_2 . Certainly, these species do not represent a complete list but were chosen also on the base of aeronomical arguments. Although, however, none of these species has been measured in the stratosphere so far, at least CH_2O is expected to be present in substantial concentrations resulting from the oxidation of methane⁶. The proton affinity of CH_2O ($165 \pm \text{kcal mol}^{-1}$) only slightly exceeds that of H_2O ($164 \pm \text{kcal mol}^{-1}$) as also proven by the qualitative observation of H_3O^+ proton transfer with CH_2O (ref. 7). The higher masses observed in our experiments also seem to fit into the concept of a stratospheric formaldehyde ion chemistry. Mass 42 ± 2 might be identified as $\text{C}_2\text{H}_2\text{O}^+$ which has already been observed⁸ in the laboratory as a product of $\text{H}^+\text{CH}_2\text{O}$ reacting with CH_2O . The masses 60 ± 2 and 80 ± 2 might be identified as the cluster ions $\text{H}^+(\text{CH}_2\text{O})_2$ or $\text{C}_2\text{H}_2\text{O}^+\text{H}_2\text{O}$ and $\text{H}^+(\text{CH}_2\text{O})_2\text{H}_2\text{O}$ or $\text{C}_2\text{H}_2\text{O}^+(\text{H}_2\text{O})_2$ respectively. The number density of the reaction partner X of PH can be estimated from the measured density ratio of NPH and PH. Assuming a chemical

Fig. 1 Fractional abundance of non-proton hydrates. ●, F33; ■, F42; ▲, S18.



equilibrium, the continuity equation

$$[\text{PH}^+] K [\text{X}] = [\text{NPH}^+] \alpha [\text{N}^-]$$

relates $[\text{X}]$ to the measured density ratio $[\text{NPH}^+]/[\text{PH}^+]$ the reaction coefficient K , the mutual recombination coefficient α and the negative ion density $[\text{N}^-]$. Taking a typical $\alpha = 5 \times 10^{-8} \text{ cm}^3 \text{ s}^{-1}$ based upon laboratory measurements⁹, a lower limit for $K = 10^{-10} \text{ cm}^3 \text{ s}^{-1}$ and a typical measured $[\text{N}^+]$ ² (which due to the absence of free electrons equals $[\text{N}^-]$) the $[\text{X}]$ profiles shown in Fig. 2 are obtained. When compared with the relative profile of the total gas density $[\text{M}]$ the $[\text{X}]$ profile steeply decreases above about 40 km as does a model profile of CH_2O (ref. 6). Even the absolute $[\text{X}]$ values, although uncertain mainly due to the assumed K and $[\text{N}^-]$ values, seem to be of the right order of magnitude. This similarity may provide further support for the identification of NPH as products of CH_2O reactions. The following simplified reaction scheme is proposed

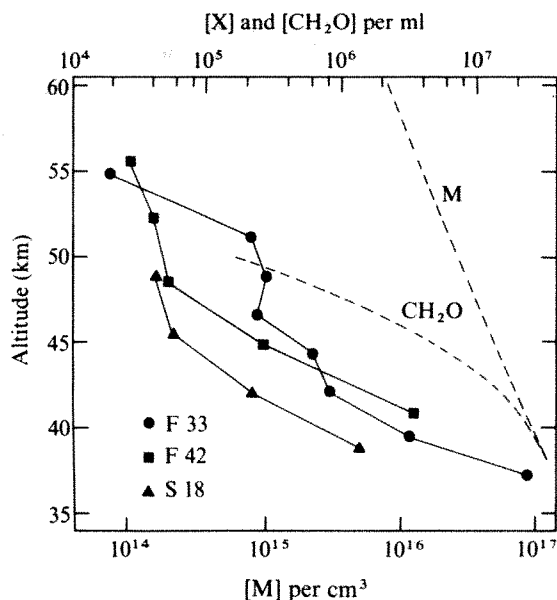


Fig. 2 Number densities of the unknown reaction partner X as derived from the data. The total gas density M (ref. 12) and a theoretical CH_2O profile⁶ are shown for comparison. ●, F33; ■, F42; ▲, S18.

(Fig. 3). The primary ions N_2^+ , O_2^+ , N^+ and O^+ which are formed by galactic cosmic radiation rapidly convert to proton hydrates. While the ion lifetime against mutual recombination is about 2,000 s or greater the PH-formation and PH-growth to an equilibrium size distribution proceeds within less than 0.01 s at 40 km. As soon as the number density of a potential reaction partner X for the PH exceeds a critical value of about $5 \times 10^6 \text{ cm}^{-3}$ (value for which $[\text{NPH}] = [\text{PH}]$) the PH-conversion becomes effective. According to measured equilibrium constants for PH (ref. 10) the most abundant PH at 40 km should be $\text{H}^+(\text{H}_2\text{O})_4$ and $\text{H}^+(\text{H}_2\text{O})_5$.

As formaldehyde not only has a higher proton affinity but also has a higher permanent dipole moment¹¹ (2.27 debye) than water (1.84 debye) it should replace H_2O when reacting with $\text{H}^+(\text{H}_2\text{O})_n$. As the difference of the CH_2O - and H_2O bond energies is probably smaller than the bond energy of the $(n-1)$ th H_2O no additional H_2O will be 'boiled off'. The product ion $\text{H}^+(\text{H}_2\text{O})_{n-1}\text{CH}_2\text{O}$ may undergo additional exchange reactions with CH_2O which may finally lead to $\text{H}^+(\text{CH}_2\text{O})_n$. Also further association of CH_2O may occur resulting in complexes $\text{H}^+(\text{CH}_2\text{O})_k$ of higher order of association ($k > n$) than the original PH. It may also be considered that the excited complex $[\text{H}^+(\text{H}_2\text{O})_n\text{CH}_2\text{O}]^*$ formed by association of CH_2O to $\text{H}^+(\text{H}_2\text{O})_n$ does not stabilise by ejection of an H_2O molecule but by ejection of molecule fragments. Cluster ions containing $\text{C}_2\text{H}_2\text{O}^+$ as a central ion might have been formed by such

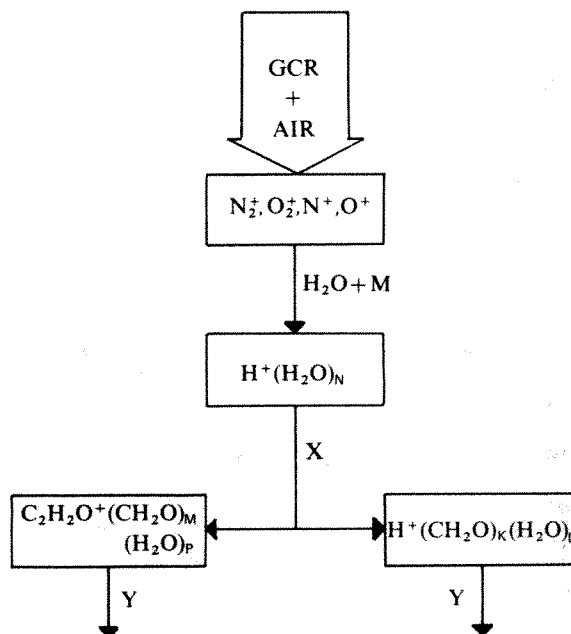


Fig. 3 Simplified reaction scheme for stratospheric positive ions at heights above 24 km. Reaction partners are indicated beside or above the arrows. M denotes an N_2 or O_2 molecule and Y denotes a molecule having a higher permanent dipole moment or proton affinity or both than CH_2O . GCR denotes galactic cosmic radiation.

processes. This was suspected previously from laboratory experiments⁸. Drift tube mass spectrometer studies carried out in our laboratory¹³ indicate that proton hydrates $\text{H}^+(\text{H}_2\text{O})_n$ with n ranging from 1 to 4 rapidly react with formaldehyde. It was also found that the product ions of the $\text{H}^+(\text{H}_2\text{O})_n + \text{CH}_2\text{O}$ process undergo a fast reaction with NH_3 resulting in $\text{NH}_4^+(\text{NH}_3)_n$ ions. Therefore an upper limit of atmospheric NH_3 at 24 km may be estimated from our stratospheric ion data. In contrast to the above estimate of CH_2O , three-body recombination has also to be considered at 24 km yielding an increased total recombination coefficient and thus an increased critical number density for a potential reaction partner of formaldehyde related ions. Taking a three-body recombination coefficient of $2.10^{-25} \text{ cm}^6 \text{ s}^{-1}$ (ref. 14) one obtains an effective recombination coefficient of $2.1 \cdot 10^{-7} \text{ cm}^3 \text{ s}^{-1}$ at 24 km. As no NH_3 related ions were found at 24 km the relative abundance of $\text{NH}_4^+(\text{NH}_3)_n$ ions was less than 0.07 corresponding to $[\text{NH}_3] \leq 7 \cdot 10^4 \text{ cm}^{-3}$.

Finally, two points should be stressed. First, that the observed ions could possibly represent fragments of ambient ions formed by electric field or shock wave-induced collisional breakup during sampling. As fragmentation causes successive stripping-off of neutral molecules (presumably CH_2O and H_2O) attached to the central ion, the nature of the central ion remains unchanged and so do the general conclusions drawn here. Second, one must consider the powerful diagnostic possibilities of stratospheric ion composition measurements for the detection of stratospheric neutral trace gases and even for a determination of their concentrations. Future *in situ*-measurements using higher mass resolution as well as laboratory studies of the relevant ion chemical processes are needed before these possibilities can be exploited.

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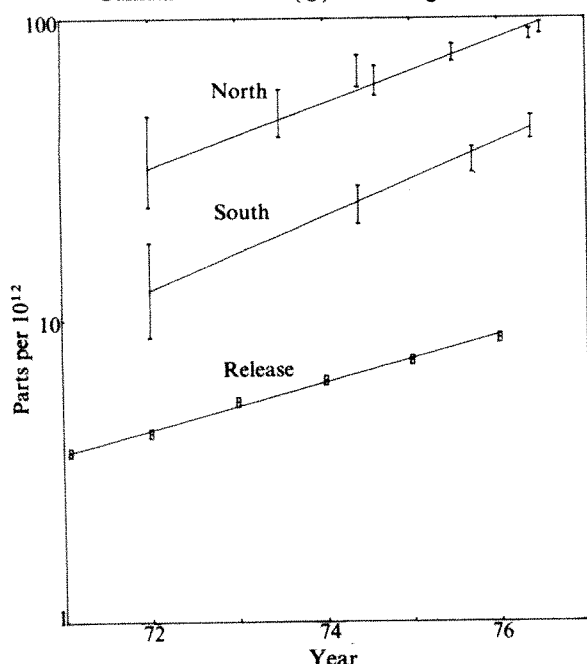
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Methyl chloroform in the troposphere as an indicator of OH radical abundance

METHYL chloroform (111 trichloro ethane) was introduced into general use in 1955-60 in response to the need for a general solvent which was less toxic than those then in use. Its ubiquitous presence in the northern and southern hemispheres, first reported in 1974 (ref. 1), has since been confirmed^{2,3}. The only significant sources of methyl chloroform are industrial releases from its use as a cleaning agent and solvent. A principal sink for its removal from the atmosphere seems to be the reaction with OH radicals. The rate of its release to the atmosphere is known with some accuracy, and consequently the measurements of its growth in the atmosphere could provide valuable information on the strength of the OH reaction sink, not only for methyl chloroform but also for other important atmospheric gases which undergo similar reactions. This communication reports measurements of the abundance of methyl chloroform made during 5 yr in the northern and southern tropospheres.

Methyl chloroform was analysed directly from 5-ml air samples using a gas chromatograph equipped with an electron capture detector. The method was that described for other halocarbons, including the fluorocarbons and carbon tetrachloride⁴. Figure 1 shows the concentration observed during the past 5 yr at measuring sites in rural

Fig. 1 Observed concentrations: parts per 10^{12} by volume of CH_3CCl_3 in the northern hemisphere (British Isles) and southern hemisphere (South Africa and Antarctica) during 1972-77. Cumulative releases (○) are in megatonnes.



regions of the British Isles, in the southern hemisphere in Cape Province South Africa and aboard the RV Shackleton. Northern hemisphere measurements were all made, except in 1972, during periods of unpolluted Atlantic air.

The observations in the southern hemisphere have been followed from just about the noise level in 1971, at an abundance of 1.2×10^{-11} , to the present level of 5×10^{-11} . The low initial finding suggests that there is no significant natural source of this compound. The absolute accuracy of the analytical method is not likely to be worse than 30% which is in general agreement with the experience of other analysts^{2,4}. Figure 1 also shows the annual releases of methyl chloroform to the atmosphere from industrial sources (personal communication from W. B. Neeley and J. Plonka). When applied to the observations here reported, using a simple two box model of the atmosphere, these data suggest an atmospheric residence time of between 5 and 10 yr. H. B. Singh (personal communication) reports observations also pointing to a longer residence time for methyl chloroform. Previous estimates of the residence time have varied from 1 to 2 yr (refs 3 and 5). It is unlikely that the measured rate of reaction of OH radicals with methyl chloroform⁶ is seriously in error, and the most likely source of the anomaly is in the time averaged value assumed for the OH concentration. The yearly average concentration is usually taken to be in the range $3-5 \times 10^9$ radicals per ml. This is a mean of several estimates⁷⁻⁹. One outstanding exception, however, is the estimate by Warneck¹⁰ of 7×10^5 per ml, for the yearly average. This lower value is more consistent with the observations reported here. The discrepancy in OH concentration is considerable and if it propagates to the reaction of the other atmospheric gases such as methane, CO and other hydrocarbons, could influence the interpretation of the chemistry of both the troposphere and the stratosphere. The comparatively uncomplicated industrial source and natural OH sink of this gas suggests that it may serve as a useful indication of the distribution of OH radicals.

Crutzen⁸ has discussed the consequences of the presence of this compound on the ozone layer. If current theories of ozone depletion by chlorine-containing species are confirmed by atmospheric measurements and if methyl chloroform is produced in ever increasing quantities for several decades, I agree with Crutzen that the atmospheric burden would give cause for concern.

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Inhomogeneities in the water properties of fogs and clouds

ALTHOUGH there is good evidence that entrainment of undersaturated environmental air exercises a profound influence upon the development of the droplet spectra in clouds, the classical description of the mixing process¹ has

Table 1 Values of droplet concentrations N , water content C , mean radius r , and dispersion S measured within hill fog (mean spacing of consecutive spectra about 3 m)

N	129	140	112	136	115	105	99	106	111	99	97	76	112	95	106	154	144	118	142	168
C	11	12	9.0	11	8.9	7.6	7.0	8.8	8.1	8.4	7.8	6.2	8.2	7.3	8.3	13	12	10	11	16
r	5.5	5.4	5.4	5.4	5.4	5.1	5.3	5.5	5.4	5.5	5.5	5.5	5.3	5.3	5.3	5.4	5.4	5.6	5.3	5.6
100S	22	29	22	26	22	32	19	24	18	25	22	20	25	23	29	25	24	21	29	29

failed to yield quantitative predictions of spectral evolution which are consistent with observation. In the classical model the mixing process is homogeneous, that is, all droplets at a given level within the cloud are, at any time, exposed to identical conditions of supersaturation or undersaturation. Recent laboratory experiments¹, however, have indicated that the mixing process in clouds may be highly inhomogeneous, with some droplets completely evaporated, or substantially reduced in size, and others negligibly influenced. This work² led to the formulation of a primitive model of inhomogeneous mixing in which it was predicted that sizeable fluctuations in the water properties of natural clouds should exist, on scales commensurate with those of the mixing process. In particular, it was proposed that there may exist closely adjacent regions in which the liquid water content was very different but the spectral shape essentially unchanged. Some recent field evidence supports this idea³ but few observational data are currently available. We describe here some preliminary results indicative of the highly inhomogeneous nature of the water properties of some clouds, on certain spatial scales.

An electrostatic disdrometer⁴ has been used to measure the size distribution of droplets within cloud and fog at

the disdrometer, of droplet concentration, N , water content, C , mean drop radius r , and spectral dispersion, S (defined as the ratio of the standard deviation to the mean size), for consecutive spectra spaced about 3 m apart in orographic hill fog. Measurements were made ~150 m above the cloud base. Since collection efficiency calibrations of the device are not complete the values of each of these four parameters, although acceptable relative to each other, are not absolute, and thus no units are attached to them. It is seen that considerable variations exist, on a scale of less than 10 m, in the water content of the fog. C varies by up to a factor of about 2.5, while the mean radius r is fairly constant. The dispersion of the spectrum undergoes significant variations, but closely spaced regions of similar values of S but widely different water content are also observed.

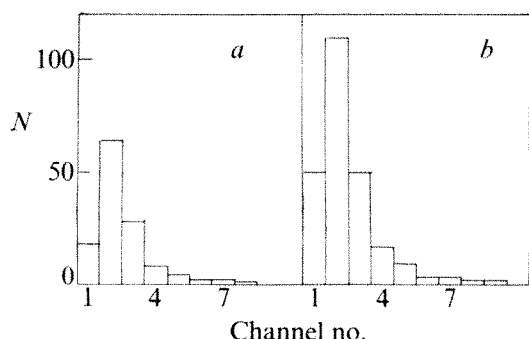
Table 2 presents a typical set of spectral parameter values measured several hundred metres above the base of a precipitating cloud, with consecutive spectra spaced about 50 m apart. Here, the fluctuations are much smaller than for the case presented in Table 1 and the measured dispersion is substantially higher. Although the liquid water content is appreciably higher than for the hill fog the mean radius

Table 2 Values of droplet concentration, N , water content, C , mean radius, r , and dispersion, S , measured within precipitating cloud (mean spacing of consecutive spectra about 50 m)

N	241	252	251	259	243	225	210	228	234	216	250	252	250	242	242	230	237	227	235	247
C	16	18	16	18	18	17	17	18	18	19	19	17	17	17	18	15	16	15	14	18
r	4.9	5.2	4.9	5.0	5.2	5.2	5.3	5.3	5.1	5.5	5.1	5.1	5.0	5.1	5.0	5.0	5.0	5.0	4.7	5.2
100S	34	30	36	35	31	34	32	31	38	29	35	34	33	31	40	32	36	35	38	32

the UMIST field-research station on the summit of Great Dun Fell (870 m above sea level) in Cumbria. Orographic effects are generally of great importance in the formation of these clouds. Droplets are sampled and sized continuously at a rate of several hundred per second with the disdrometer, which can be mounted either on a platform close to the ground or on a 30 m mast. The level of spatial resolution of the water properties of the clouds is dependent on the horizontal wind speed, which was measured. In light winds statistically meaningful spectra can be obtained at intervals down to several metres; and in winds of around 25 m s^{-1} , characteristic of this site, the minimum spatial scale that can be studied is some tens of metres.

Table 1 presents a typical set of values, obtained with

Fig. 1 Characteristic droplet spectra measured in (a) orographic hill fog, (b) precipitating cloud. N is the droplet count. The channels are $0.9 \mu\text{m}$ wide, and channel 2 corresponds to a radius of $5.2 \mu\text{m}$.

is significantly lower (by about $0.3 \mu\text{m}$). This could be a consequence of preferential capture by raindrops of the larger droplets within the condensate spectrum.

Figure 1 presents characteristic uncorrected spectra obtained for the two situations covered by Tables 1 and 2. In both cases the principal contribution to the water content of the spectrum is provided by droplets in a narrow central size band; the contribution from the largest droplets in the spectrum is very small. Statistical analysis shows that fluctuations in water content observed cannot be a consequence of randomness in rate of arrival of droplets; this conclusion was confirmed in laboratory tests using a cloud tunnel.

Preliminary results obtained so far indicate that, on certain spatial scales at least, very considerable fluctuations exist in the water properties of clouds and fogs, and we hope that future work will provide more quantitative information on the nature of these inhomogeneities in a variety of meteorological conditions.

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Fine structure in caustic junctions

EVERYONE has seen the changing patterns of bright caustic lines traced on the sea bed or on the bottom of a swimming pool^{1,2}. The caustics are formed when sunlight is focused after refraction by smooth waves on the water surface; similar caustics can result from reflection. Here we draw attention to one feature of these patterns: the frequent occurrence of junctions at which three caustics appear to meet (Fig. 1).

It might seem that this is not worthy of comment—after all, the stability of triple junctions under perturbation makes them widespread in many natural systems of lines and surfaces^{3,4} such as mud cracks, foams, and the markings on giraffes. But triple junctions in caustic patterns would be surprising. The reason is that, except in special circumstances, the forms of caustics are restricted to be 'elementary catastrophes'⁵⁻⁶ and in two dimensions (i.e. two 'control variables') these are smooth curves ('fold' catas-

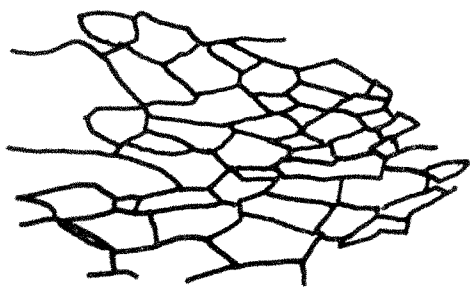


Fig. 1 Caustics formed by a wavy water surface (after Minnaert¹).

trophenes) which may have cusp points: triple junctions occur nowhere in the list of elementary catastrophes. The qualification 'except in special circumstances' implies the assumption of genericity that underlies catastrophe theory. It means in this case the absence of high symmetry in the wavefront whose normals envelop the caustic and is certainly satisfied for the random water waves considered here.

These considerations suggest that the triple junctions are illusory. In fact they are the appearance under low resolution of a caustic structure with fine detail on several levels. The following argument shows that this structure involves the meeting of six lines rather than three. Below each

Fig. 2 Line pairs where caustic surfaces from ridges intersect the sea bed.

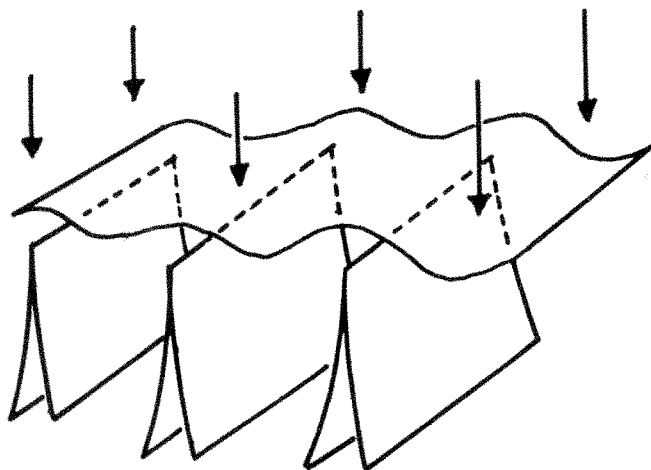


Fig. 3 Glass 'lollipop' on whose surface three grooves meet.

'ridge' on the water surface is a caustic surface with two sheets that meet along a cusped edge (Fig. 2); if the sea bed intersects this surface it will generally do so in two almost-parallel lines (these are easily seen in sunlight in, say, 2 m depth of water on which the waves are long-crested). The ridges meet in threes on the water surface, and the associated meetings of three line pairs on the sea bed are the 'triple junctions' under discussion.

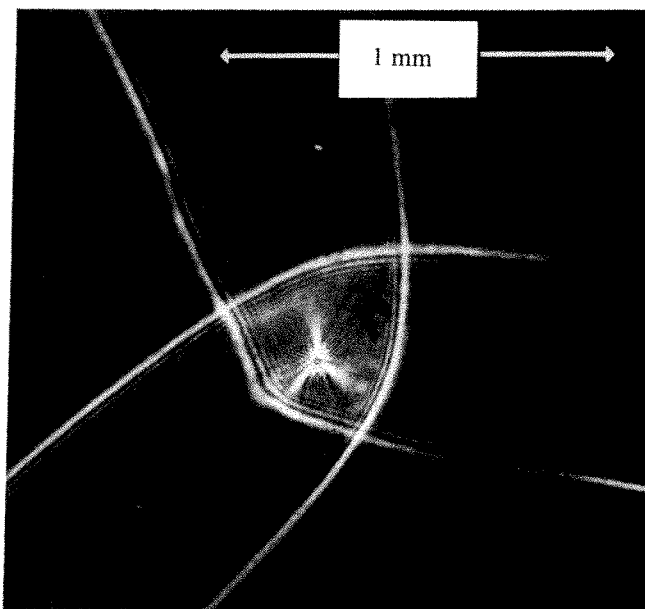


Fig. 4 Typical fine structure in caustic junction, formed by refraction of white light from a small distant source through the 'lollipop' of Fig. 3 and photographed with the aid of a microscope.

To study the details of such a junction 'in vivo' would be difficult in view of its rapid motion and the blurring caused by the 0.5° divergence of rays from different parts of the Sun. Therefore conditions near the junction of three ridges were simulated 'in vitro' on the surface of the disk of a glass 'lollipop' (Fig. 3) formed in the molten state. The diameter of the disk was 40 mm. White light from an illuminated pinhole was viewed through a microscope after being refracted by the disk. (It was easier to produce grooves on

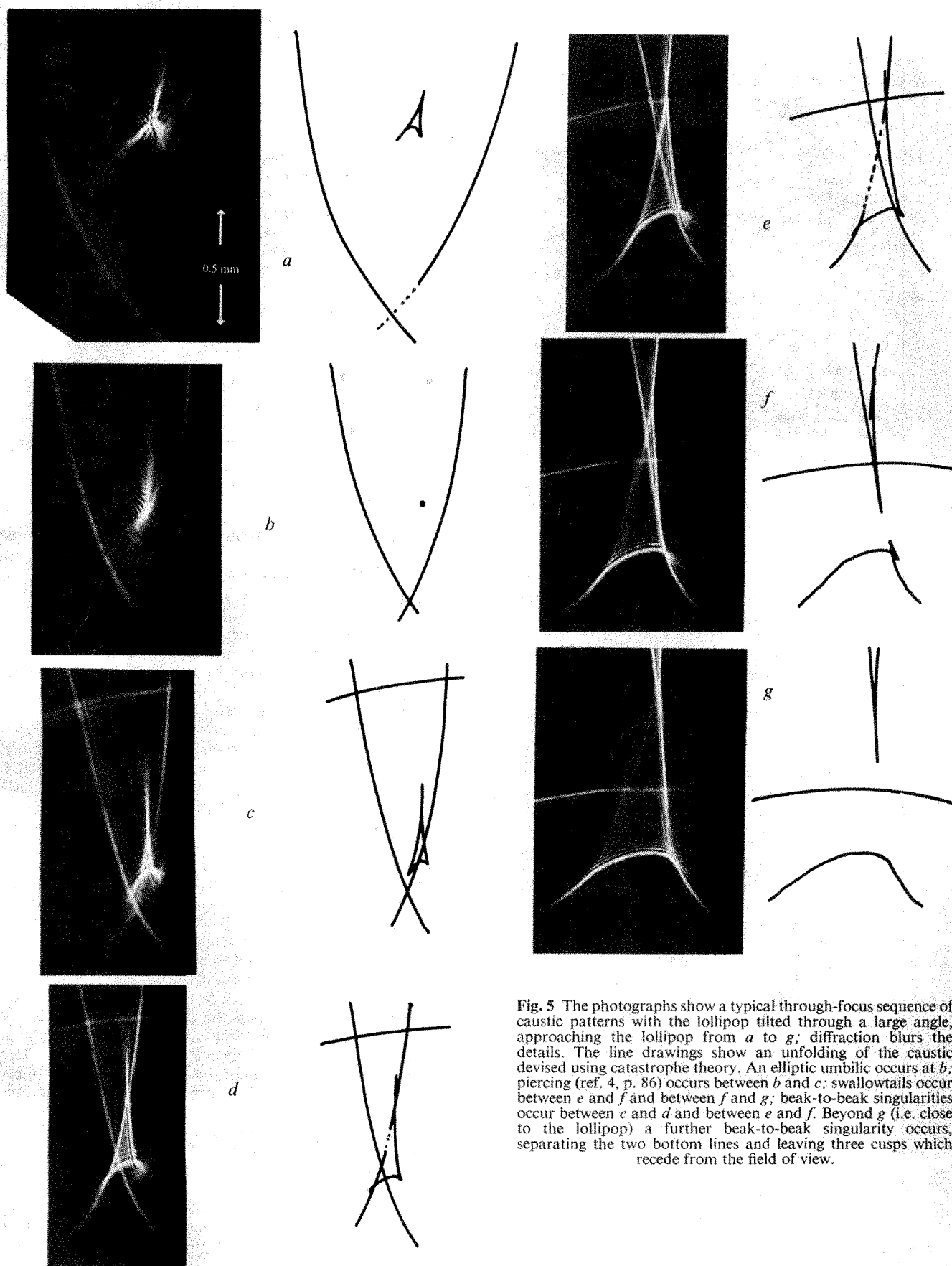


Fig. 5 The photographs show a typical through-focus sequence of caustic patterns with the lollipop tilted through a large angle, approaching the lollipop from *a* to *g*; diffraction blurs the details. The line drawings show an unfolding of the caustic devised using catastrophe theory. An elliptic umbilic occurs at *b*; piercing (ref. 4, p. 86) occurs between *b* and *c*; swallowtails occur between *e* and *f* and between *f* and *g*; beak-to-beak singularities occur between *c* and *d* and between *e* and *f*. Beyond *g* (i.e. close to the lollipop) a further beak-to-beak singularity occurs, separating the two bottom lines and leaving three cusps which recede from the field of view.

the glass surface rather than ridges, so that the caustics were virtual rather than real.) In this way the three line pairs were easily resolved and typical patterns do indeed show the smooth curves and cusps anticipated on the basis of catastrophe theory (see Fig. 4). Note the inner cusped

'triangle', for this provides the key to the understanding of the higher-dimensional caustic structures now to be discussed.

Altering the focus of the microscope introduces an additional control variable which enables the three-dimensional

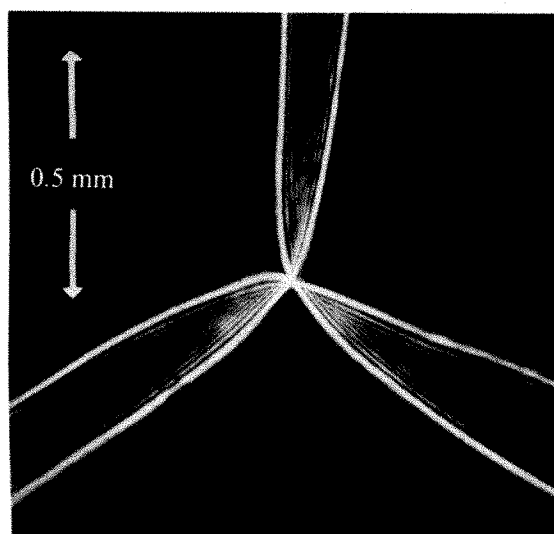


Fig. 6 Singular section of a higher catastrophe.

caustic surface to be explored in two-dimensional sections. In nature this new variable could represent time. A 'through-focus' sequence of patterns is shown on Fig. 5a-g. The complicated changes in caustic structure must be governed by catastrophe theory with three control variables. In our experiments the finest details of some of these changes were obscured by characteristic diffraction effects; the intensity and linear scale of the diffraction effects increase, for fixed wavelength, with the order of the singularity. According to catastrophe theory, cusp points may appear and disappear in pairs, either at beak-to-beak or lip singularities^{4,5} (where the plane of focus touches a cusped edge of the caustic surface) or at swallowtail catastrophes (singular points of the caustic surface). Guided by these principles it was possible to devise a series of catastrophes (the line drawings on Fig. 5) to account for the transformations on the photographs. The line drawings show the patterns that would be formed in the limit of geometrical optics where the wavelength approaches zero. There are further singular points of caustic surfaces, associated with elliptic and hyperbolic umbilic catastrophes, but since these catastrophes are symmetric in their unfoldings they do not result in changes in the topology of the two-dimensional patterns; an elliptic umbilic occurs on Fig. 5b as the cusped 'triangle' shrinks to a point and expands again. All this behaviour is typical (generic): no special care is required in orienting the lollipops or in manufacturing them (other than making them smooth). Qualitatively identical phenomena were also observed in reflection.

The tilting of a lollipop provides two more control variables, θ_1 and θ_2 , say. By changing θ_1 it is possible to find a through-focus sequence where the cusped triangle shrinks to a point just as it reaches one of the caustic lines; this is a parabolic umbilic catastrophe point P on the three-dimensional caustic hypersurface in the four-dimensional control space C. The sequence on Fig. 5 constitutes a three-dimensional section S of C, and the fact that P lies close to S explains the small scale of detail in the patterns. In Thom's terminology⁴ the parabolic umbilic is the 'organising centre' for caustics near P and its unfolding justifies the sequence of patterns in the line drawings of Fig. 5. It is also possible to make two swallowtails coincide; this is a butterfly catastrophe. Finally, by also changing θ_2 this butterfly and parabolic umbilic can be made to coincide at one stage in the through-focus sequence; the two-dimensional pattern (Fig. 6) is then a singular section of

the higher-order catastrophe, presumably involving five control variables, which organises all the triple junction caustic patterns discussed here.

We draw two conclusions from this study. First, nature's line patterns are not all of the same sort; the triple junctions generic in mud cracks cannot occur with caustics. Second, the geometrical optics of cylindrically symmetric artefacts such as telescopes, where departures from the ideal point focus are treated as 'aberrations', is very different from the geometrical optics of nature, where the generic forms of caustic surfaces are governed by the mathematics of catastrophe theory.

We thank Mr Tony Osman for making the 'lollipops'.

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Polarised infrared cathodoluminescence from platelet defects in natural diamonds

WE report here that the large platelet defects occasionally found in natural diamonds emit polarised cathodoluminescence in the near infrared. Lattice defects in natural diamonds attract as much scientific enquiry today as at any time since the anomalous 'spike' diffuse X-ray reflections from single-crystal diamonds were discovered¹. That platelet-type defects segregated on {100} planes of the diamond matrix could well account for the 'spike' X-ray reflection observations^{1,2} was shown by Frank³. Transmission microscopy⁴ revealed just such platelets, with diameters generally in the range 10 to 100 nm, profusely present within the most common form of diamond (Type Ia). Mendelssohn⁵ described a specimen containing platelets on {100} that were more than two orders of magnitude greater in diameter than those that had become well known through transmission electron microscopy. The large platelets emitted a green-yellow cathodoluminescence which made them conspicuous when they lay not deeper below the specimen surface than the penetration range of the bombarding electron beam ($\sim 10 \mu\text{m}$). Work in our laboratory has shown that platelets on {100} span the gamut of sizes from the largest observed by Mendelssohn down to the more familiar sub-micrometre objects seen electron-microscopically. This knowledge has been acquired by combining X-ray topography (using both normal Bragg reflections and the anomalous 'spike' reflections), cathodoluminescence topography, ultraviolet absorption topography and transmission electron microscopy at 100 kV and 1 MeV (refs 6,7). Platelets exceeding 5-10 μm in maximum dimension are rare compared with those of sub-micrometre size; but our experience affords us a good chance of identifying solely by X-ray topography regions within diamonds where there reside platelets having extreme dimensions exceeding 1-2 μm (and such platelets we dub 'giant').

We conclude⁶ that the principal visible emission from individual platelets is the 'H3' system^{8,9}; although in our experiments (so far done only at or slightly above room temperature) we have not yet succeeded in spectrographically recording the 2.46 eV zero-phonon line in the weak spectra of single platelets. The polarisation of the visible cathodo-

luminescence from platelets⁶ is notable: the measured ratio of intensity emitted with **E** vector in the platelet plane to that with **E** vector normal to the platelet being about 10.

There is much uncertainty regarding composition and structure of the platelets¹⁰⁻¹². Apart from expressing dissent with the view of Evans¹³ and Woods⁷ that the platelets are composed of interstitial carbon atoms, we will not debate this topic since our concern is to present new findings on the optical properties of platelets. The discovery that cathodoluminescence from giant platelets can be seen in the near infrared using an image converter has been briefly reported⁶. This observation has been followed up by photographic recording with Kodak High Speed Infrared film. It has now been discovered that the infrared emission from platelets is polarised with **E** vector in the platelet plane, but with a considerably higher polarisation ratio than in the case of their visible emissions.

The cathodoluminescence topographs in Fig. 1 (taken by means similar to those previously described¹⁴) show part of the specimen C19 studied by Mendelsohn. Here platelets range up to 40 μm in extreme dimensions. Differing brightnesses of platelets which lie in the cube plane nearly parallel to the specimen surface result from their differences in depth below the surface; these images are unaffected by rotation of the Polaroid filter. (In this specimen fan-shapes and kite-shapes predominate among the largest platelets; in other specimens elongated shapes aligned randomly along one or other of the two $\langle 110 \rangle$ axes in the platelet plane are more common.) The appreciable width of edge-on platelet images arises from shortcomings of the optical system which was not designed for infrared use. Contrast between platelets and matrix is far superior to that in the visible region where platelet emission has to compete with the low-energy tail of the cathodoluminescence continuum known as Band A (refs 9, 15). To facilitate comparison of features in Fig. 1, a Polaroid filter giving only partial extinction at infrared wavelengths was used. In experiments

undertaken to assess the degree of polarisation of the infrared emission we used Polaroid Type HR linear polariser which is highly effective at the longest wavelengths (about 900 nm) recordable by Kodak High Speed Infrared film. Despite a sixfold increase in electron beam current per unit area of specimen as well as reductions in optical magnification so as to maximise image brightness of the polarisation component attenuated by the Polaroid, we could determine only a lower limit, about 40, of the ratio of intensity of radiation with **E** vector parallel to the platelet plane to that with **E** vector normal to the platelet. Our results reinforce our earlier conclusion⁶ that we are observing the emission band peaking at 1.25 eV (990 nm) described by Wight *et al.*¹⁶ which correlates well with the infrared absorption at 170 meV (7.3 μm) generally accepted as a measure of total area of the common, sub-micrometre platelets in unit volume of specimen. The high degree of polarisation of platelet infrared emission constitutes a well-defined optical characteristic that any model for platelet structure (and for optical processes associated with platelets) must satisfactorily accommodate.

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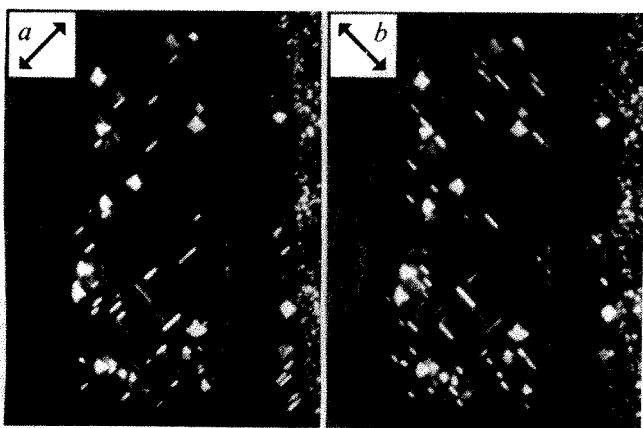


Fig. 1 Infrared cathodoluminescence from large platelets lying on cube planes in a natural diamond. The specimen surface is polished about 5° off (001) orientation: hence platelets close to the specimen surface and lying in (001) are seen nearly face-on. The direction $[110]$ points horizontally to the right. Platelets parallel to (100) are seen edge-on as bright lines running from top right to lower left, and those parallel to (010) as bright lines running from top left to lower right. Field width is 0.5 mm; the length of the arrows indicating **E** vector directions is 100 μm . Electron beam energy 20 kV, specimen current 1.1 $\mu\text{A mm}^{-2}$. Photographed on Kodak High Speed Infrared film through the visually opaque, infrared transmitting Kodak-Wratten filter 87C and through a Polaroid filter used for visible wavelengths. The lighter areas and strips seen trending roughly vertically on either side of the field are due to numerous smaller platelets not fully resolved individually which reside in crystal growth layers parallel to $(\bar{1}\bar{1}1)$ on the left and to $(11\bar{1})$ on the right. *a*, Polaroid rotated to transmit the **E** vector lying in the plane (100); *b*, lying in (010), as indicated by arrows.

Supported ^{242}Cm in the marine environment

BOWEN and Livingston¹ have reported the presence of ^{242}Cm (half-life 163 d) in two samples of sea water which had been kept in sealed containers for 12 yr since the time of collection. Because the ^{242}Cm originally present in the samples would have decayed completely after this period of storage, they suggested that the ^{242}Cm which they detect is supported by a long-lived precursor, $^{242\text{m}}\text{Am}$; this radionuclide would not itself be detectable in these samples because of its much longer half-life (152 yr). They invited other workers in radiochemistry to search for similar evidence for $^{242\text{m}}\text{Am}$ in the environment, and we have done so in some samples collected from the vicinity of the fuel-processing plant at Windscale where controlled discharges of transuranic nuclides to the marine environment are permitted under the provisions of the United Kingdom Radioactive Substances Act, 1960. The radiological implications of these discharges have been discussed by Hetherington *et al.*²

We obtained a sample on 15 December 1974 from the area of the processing plant and extracted the actinides from the sample matrix by strong acid digestion in oxidising conditions. After reduction with sulphur dioxide they were concentrated by coprecipitation, first on calcium oxalate and then on 5 mg of added neodymium carrier. Am, Cm and Nd were then separated from the Pu by anion exchange

in 9 M HCl and further purified by precipitation as fluoride in acid solution, followed by conversion to the hydroxide. Am and Cm were then separated from Nd by anion exchange in a 4 M NH_4SCN medium at 80 °C and electro-deposited on to a stainless steel disk from an $(\text{NH}_4)_2\text{SO}_4/\text{H}_2\text{SO}_4$ medium. ^{243}Am was used as a yield tracer.

The separated source of Am and Cm was first measured on 24 July 1975 by α -spectrometry and was then measured at intervals over a period of some 500 d to follow the decay of ^{242}Cm . The results are shown in Table 1.

Table 1 Decay of separated ^{242}Cm

Date	Decay period (d)	^{242}Cm (pCi)	^{241}Am (pCi)
24 July 1975	0	48.04 ± 1.79	43.1 ± 1.6
14 Jan. 1976	174	23.47 ± 0.36	—
17 May 1976	298	14.32 ± 0.27	—
12 Jan. 1977	538	5.07 ± 0.28	—

The $\pm$ figures denote σ error on counting only. Data are corrected for Am yield.

A regression on these data by the method of least squares produced a derived half-life value of 166.0 ± 2.1 (1 σ) d, which is very close to the accepted value for ^{242}Cm of 163 d; the coefficient of determination (r^2) was 1.00. If the half-life was taken as 163 d, however, and the assumption then made that the apparent longer half-life was due to a small, constant amount of ^{242}Cm , supported by a precursor with very long half-life, then the decay equation could be restated

$$(y-d) = K \exp(-0.693/163)t$$

where y is measured activity (total) of ^{242}Cm (pCi), d is supported ^{242}Cm (pCi), t is time of decay (d) and K is unsupported ^{242}Cm at time zero (pCi).

This equation can be arranged

$$y = d + K \exp(-0.693/163)t$$

A linear regression can again be performed with $\exp(-0.693/163)t$ as the x -value and the measured ^{242}Cm activity as the y -value. The value of d (the equilibrium value of supported ^{242}Cm) obtained in this way was 0.60 ± 0.32 (1 σ) pCi, compared with the ^{241}Am value of 43.1 ± 1.6 pCi. Thus the ^{242}Cm is $1.36 \pm 0.73\%$ of the ^{241}Am : this is significantly different from zero ($P=0.03$).

The analysis of other samples in the Windscale area has not included counting at intervals to measure decay of ^{242}Cm . But, following the procedure of Bowen and Livingston, Am and Cm were recently separated from some marine sediment samples collected in April 1973, some seven half-lives of ^{242}Cm after collection; in none of them did the total ^{242}Cm exceed 0.2% of the ^{241}Am , and the supported $^{242}\text{Cm}/^{241}\text{Am}$ in these samples can therefore be stated unequivocally to be $\geq 0.2\%$.

The results of the investigation indicate that little, if any, supported ^{242}Cm has been detected in this area but further work is in hand to check these preliminary data.

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Are rare earth elements mobile during spilitisation?

FROM the study of rare earth elements (REE) in basalts, spilites and hydrothermally altered basalts in the ocean floor^{1–5}, ophiolites^{6,7}, geosynclines^{3,8} and low-grade to greenschist facies regions⁹ it has come to be accepted that the REE are immobile during rock alteration. This conclusion has led to the use of certain abundance ratios, particularly La/Sm and La/Yb, as indicators of parent magma type and genesis. Despite some suggestions^{2,10} that REE, such as La and Ce, may be mobile during sea-water/rock interaction, hydrothermal alteration or spilitisation of igneous rocks, the REE continue to be treated (e.g. refs 6 and 7) in an analogous fashion to Ti, Y and Zr (ref. 11). Here we show from analyses of a variably altered tholeiite flow that spilitisation may, in some circumstances, affect the REE abundances and their chondrite normalised pattern.

One major obstacle to the study of the relative stability of REE abundances during rock alteration is the rarity of outcrops that show a continuous gradation from a fresh to a highly altered condition. The Bhoiwada section, part of a volcanic sequence on Bombay Island, India, is such an occurrence and allows a test to be made of possible mobility of REE during spilitisation.

The Bhoiwada section exhibits^{12–15} the gradation of a fresh, black, plagioclase-clinopyroxene tholeiite into a green, albite-chlorite-zeolite spilite within a single Deccan flow unit, 30 m thick (Fig. 1). Details of petrography, major and minor element analyses and field description are provided by Vallance¹⁴ and Sukheswala¹². Vallance¹⁴ also presents evidence to show that the section represents one flow unit, part of which may have been subaerial and part subaqueous. The section can be divided into five zones¹⁴. Only zone 5 is pillowed; we have subdivided an outer margin of a pillow into three parts (Fig. 1b). The pillows are often highly amygdaloidal and contain rosettes of laumontite, with prehnite, quartz and calcite. There is a concentration of amygdales immediately below the thin (less than 5 mm thick) black, chlorite-rich rind. Brief mineralogical descriptions for each sample are given with Fig. 2.

REE abundances in powdered rock samples were determined by instrumental neutron activation analysis¹⁶. Accuracy and precision are estimated to be about 4% at the 95% confidence level, for all REE except the Ho data which are about 10%.

REE abundances for the five zones are presented as chondrite normalised diagrams in Fig. 2 (a) and Fig. 3. La/Sm and La/Yb ratios are given in Table 1. For comparison, REE patterns are shown (Fig. 2) for two of Kuno's Japanese basalt types¹⁷, an alkali basalt and nepheline benmoreite from the Dunedin volcano, New Zealand¹⁸, and a tholeiite from the neovolcanic zone in

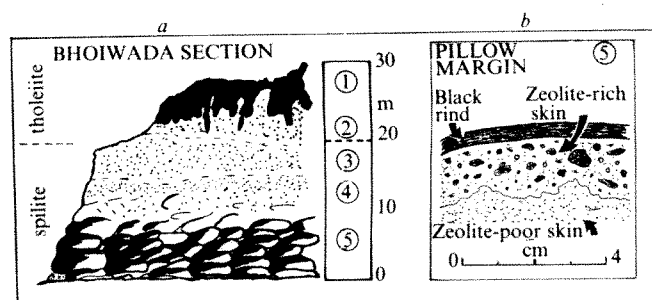


Fig. 1 a, Sketch of Bhoiwada section and its five zones (after Vallance¹⁴ and Sukheswala¹²); b, Sketch of pillow margin, zone 5.

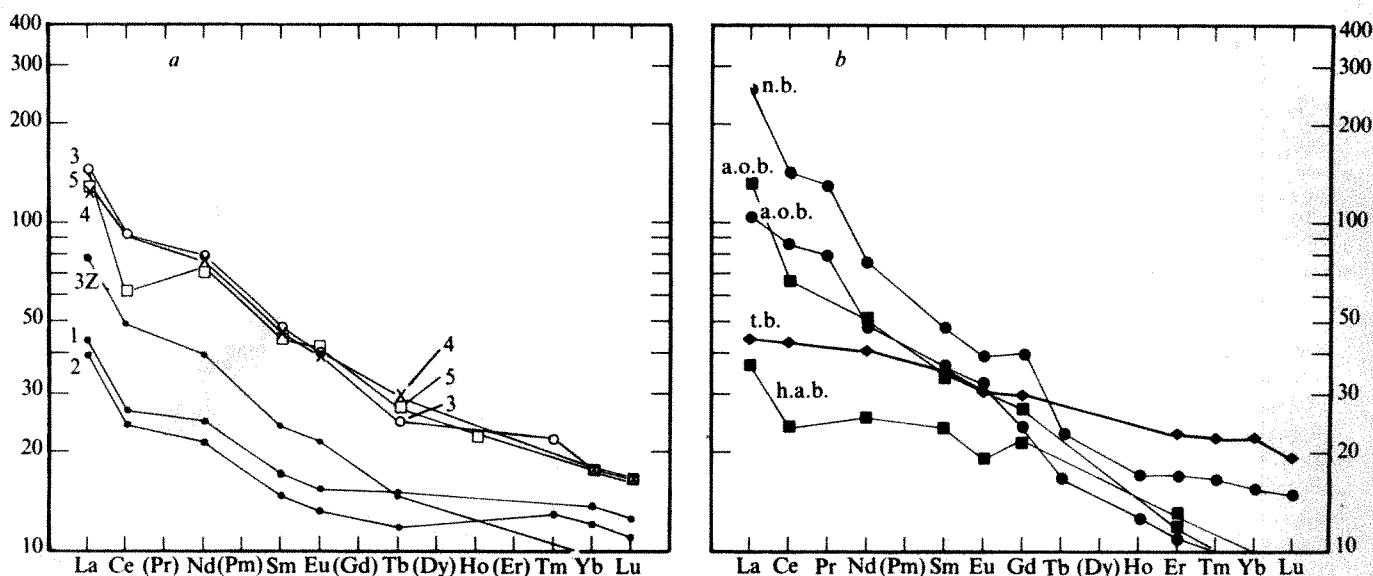


Fig. 2 REE abundances (chondrite normalised after Haskin *et al.*²⁴) from *a*, Bhoiwada, zones 1-5. Sample descriptions: 1, plagioclase-clinopyroxene-(magnetite) tholeiite. Some phenocrystal plagioclase and clinopyroxene. Intersertal patches of altered glass. 2, Same as 1 except for appearance of small chlorite-filled amygdaloids and chloritised matrix. 3, (open circles) Chlorite-albite-prehnite-spilite. 3Z: Strongly zeolitised spilite. Laumontite-prehnite-quartz amygdaloids within a chlorite-albite matrix. 4, (crosses) Chlorite amygdaloids within albite-chlorite-spilite. 5, (open squares) Core of pillow. Albite-chlorite-prehnite-spilite. Amygdaloids of chlorite and prehnite. All spilites possess relict clinopyroxenes. *b* Fresh recent lavas: ■, Japan—high-alumina basalt (h.a.b.) from Fuji volcano, Mishima and alkali olivine basalt (a.o.b.) from Oki Dogo Island¹⁷. These two basalts are representative of two of Kuno's basalt types. ●, New Zealand—alkali olivine basalt and nepheline benmoreite (n.b.) from the Dunedin volcano, East Otago¹⁸. ◆, Iceland—tholeiite basalt (t.b.) from Askja caldera, neovolcanic zone¹⁹.

Iceland¹⁹. La/Yb ratios are listed for several basalt types from different environments in Table 2.

The marked increase in the concentrations of REE with spiliteisation can be seen easily from Figs 2 and 3. Not only do absolute abundances change but also the La/Sm and La/Yb concentration ratios (Table 1). The observed

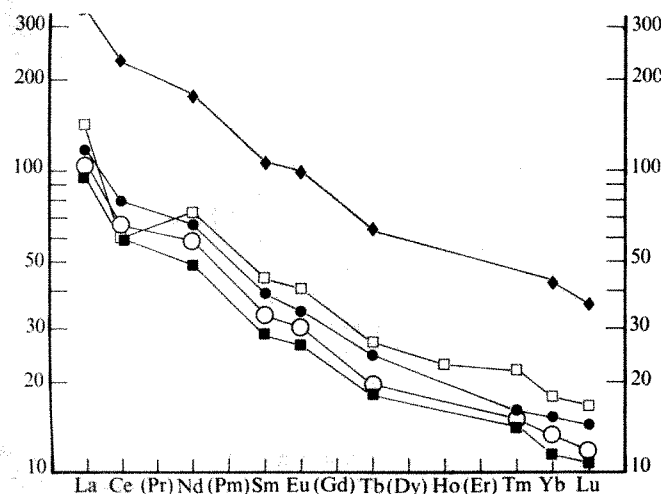


Fig. 3 Chondrite normalised REE abundances within a single pillow—zone 5 (see Fig. 1). ◆, black rind; □, core; ●, zeolite-free skin; ○, zeolite-rich skin; ■, 15087B—a pillow skin donated by Vallance.

changes are consistent with the generation of spilites as being a secondary process¹⁴⁻¹⁵. However, it is to be noted that there is virtually no change in the ratios of the values observed in slightly altered and more extensively altered material.

These results have implications for former studies in which REE abundance patterns of altered basalt and spilites were compared with those of fresh rocks. For example, the pattern of a zone 5 pillow alone might have been interpreted as the spiliteised equivalent of an alkali basalt (see Figs 2*b* and 3). The shift in La/Yb ratios

transcends values from numerous basalt types and geological environments (Tables 1 and 2). The variation also mimics natural basalt crystallisation-differentiation patterns. Consequently our results for the specific case of a light REE (LREE) enriched pattern at Bhoiwada indicate that it is unwise to interpret altered basalts as possessing alkaline affinities on the basis of the REE abundances alone.

Two examples of such interpretations are: the REE patterns shown by rocks of a lower Palaeozoic greenstone terrain in West Norway, indicate different degrees of partial melting of the source material for parent magma⁹; and the suggestion⁷ that the genesis of a Mesozoic ophiolite, exposed in central Greece, involved the existence of a tensional rift system. Our data show that an alternative feasible interpretation is that the REE patterns have resulted partly from secondary metamorphic or metasomatic activity. A similar interpretation¹⁰ has been made for some zeolitised Icelandic lavas.

The layer silicates, such as chlorite, and the low-birefringent, very fine-grained matrix in the spilites, could play a significant role in determining the REE abundance patterns. Roaldset and Rosenquist²⁰⁻²² describe chlorite in Quaternary clays of the Numedal area, Norway, as capable of increasing the La content by almost four times, through

Table 1 La/Sm and La/Yb weight ratio variation within the five zones of Bhoiwada section

Zone	La/Sm	La/Yb
1	4.7±0.2	5.4±0.2
2	4.9±0.2	5.5±0.2
3	5.9±0.3	14.0±0.6
3 (strongly zeolitised)	6.1±0.3	13.5±0.6
4	5.2±0.2	12.4±0.5
5	6.0±0.3	13.4±0.6
a, black rind	5.7±0.3	13.0±0.6
b, skin—zeolite rich	5.6±0.3	12.9±0.6
c, skin—zeolite poor	5.9±0.3	13.2±0.6
d, core	6.1±0.3	14.3±0.6
e, skin—15087B		

Table 2 Common La/Yb weight ratios from different environments

	La/Yb
Hawaiian tholeiitic series ¹	3.9–7.0
Iceland tholeiite ¹	2.7–7.2
DSDP Leg 15, site 15 ¹	6.2–7.5
Hawaiian alkali series ¹	8–15
Calc-alkaline extrusives ^{2,3}	6–8
Island arc tholeiites ^{2,3}	1–2

adsorption. Their studies also suggest that such adsorption processes raise significantly both the La/Sm and La/Yb ratios.

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540-Myr-old anorthosite complex in the Grenville Province of Quebec, Canada

MASSIF-TYPE anorthosites and related rocks are an important component of the Grenville and Nain structural provinces of the Canadian Shield (Fig. 1), as well as in high grade metamorphic terrains in other parts of the world. There are many outstanding problems in the study of these rocks, not least of which is their age. Most geochronological data have been obtained from the associated granitic rocks of this suite as anorthosite itself contains very little rubidium and zirconium. Because of post-crystallisation deformation, however, the relationship between the anorthosite and the

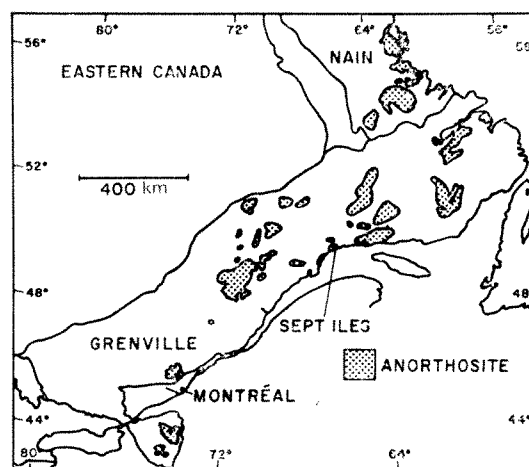


Fig. 1 The location of the Sept Iles and other major anorthosite complexes in the Grenville and Nain Provinces are shown. The northern boundary fault of the St Lawrence Graben follows the shore line in most of this area.

associated rocks is often tenuous and frequently disputed. Within the Grenville Province metamorphosed rocks believed to be genetically associated with anorthosites have yielded ages ranging from 1,020 to 1,130 Myr (refs 1–4). The high-grade of metamorphism surrounding the Morin and Lac St Jean anorthosites is believed by some researchers to be due to anorthosite emplacement. These 'aureoles' yield Rb–Sr ages^{5,6} of about 1,500 Myr. Anorthosite complexes in the Nain Province are undeformed, and the adamellite of the suite has yielded a U–Pb zircon age⁷ of about 1,300 Myr. Barton⁸ obtained a Rb–Sr isochron age of 1,418 Myr from a digested inclusion in the Nain anorthosite. Here we present geochronological data from the undeformed Sept Iles anorthosite, and show it to be Cambrian in age.

The Sept Iles anorthosite complex is situated on the north shore of the Gulf of St Lawrence, about 800 km north-east of Montreal, on the southern edge of the exposed portion of the Grenville Province. Apart from rare alkalic rocks, ages of Grenville Province rocks exceed 900 Myr. The Sept Iles anorthosite complex is, however, situated on the boundary between the St Lawrence Graben and the Grenville Province, which is the locus of intrusion of alkalic rocks ranging in age from early Cambrian to Cretaceous⁹. Its boundaries are clearly indicated on aeromagnetic maps, and by a substantial positive gravity anomaly measuring approximately 50 km × 70 km. Its host rocks on shore are thought to be a moderately deformed anorthosite. The complex has been considered to be a good example of undeformed Grenville anorthosite¹⁰. The islands have been mapped in detail (S. T. Ahmedali, personal communication) and consist of anorthosite grading upward into well layered gabbro. The gabbro is overlain along a sub-horizontal contact near sea level by granitic rocks ranging from partly layered monzonite near the base to adamellite. The granitic rocks are slightly alkaline in their chemistry and contain alkali pyroxenes and amphiboles. Field relationships show clearly that the more basic members of the suite were only partly consolidated during the emplacement of the monzonite. An age from this unit would therefore date the anorthosite. Verification of this relationship was obtained by isotopic means from more rubidium-rich segregations within the anorthosite itself.

Isochrons have been obtained from a single outcrop of the layered monzonite and from a 500-m section through the adamellite. These yield ages of 538 ± 17 Myr and 552 ± 12 Myr (Fig. 2a, b) (all errors are expressed as single standard deviations). Rb–Sr data have also been obtained

from alkali rich patches within the anorthosite. The contemporaneity of these bodies and the anorthosite is clearly visible in the field, because of the lack of deformation (Fig. 3). The host anorthosite consists of black plagioclase crystals 1 to 4 cm in length, with small amounts of interstitial mafic material. As the pegmatite is approached the grain size of the plagioclase increases, and these large crystals project into the zone of micrographic orthoclase and quartz. These patches are less than 1 m in diameter and are frequently aligned along what are assumed to have been zones of low pressure in the semiconsolidated anorthosite magma. More conventional anorthosite pegmatites also occur in zones between the orthoclase rich areas.

The isochron of Fig. 2c is from two adjacent pegmatites and their host anorthosites. The points define an age of 555 ± 12 Myr and an initial ratio of 0.7036 ± 0.0004 . The second isochron (Fig. 2d) is derived from three samples of a single pegmatite, combined with a single sample from a

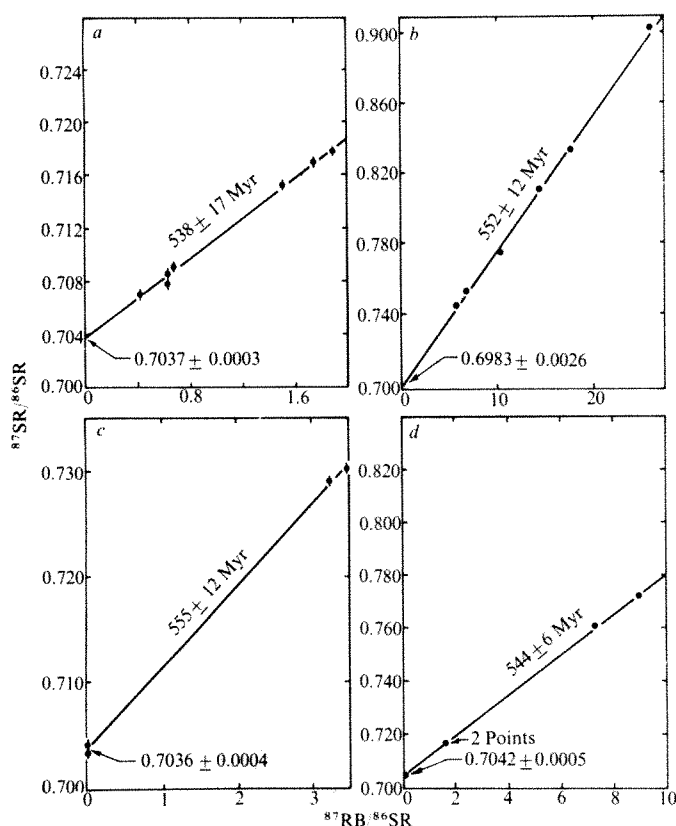


Fig. 2 Isochrons from the Sept Iles anorthosite and related rocks. The low initial ratio of (b) could be due to the abundance of xenoliths in this unit. Error bars (single s.d.) were calculated for both variables but are only shown where they exceed the diameter of the symbol. Ages were calculated using $\lambda^{87}\text{Rb} = 1.39 \times 10^{-11} \text{ yr}^{-1}$. a, Monzonite; b, adamellite; c, two adjacent pegmatites; d, three samples of a single pegmatite, combined with a single sample from a nearby pegmatite and the host anorthosite.

nearby pegmatite and the host anorthosite. The points are within experimental error of a line representing an age of 544 ± 6 Myr and an initial ratio of 0.7042 ± 0.0005 .

The initial ratios of these isochrons are consistent with ratios of 0.7039 (s.d. 0.0005) obtained from eight anorthosite and gabbro samples distant from the rare pegmatitic areas.

All ages reported here agree within narrow limits of experimental error, supporting the temporal relationships observed in the field. Granite, syenite, and carbonatite



Fig. 3 The alkali-rich pegmatite shown here is located near the pegmatites used to construct isochron 2c. The large black plagioclase crystals that project into the micrographic zone are clearly visible.

bodies associated with the St Lawrence Graben system yield similar ages^{9,11}. The adamellite of the Sept Iles complex is very similar to some of these rift-related rocks. The location of anorthosite in a rift environment, associated with acidic alkaline rocks, may provide insight into the origin of both these suites. The results also suggest that many anorthosites may be anorogenic, although some may be deformed by later orogenic events.

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Phenology of sound-producing arctiid moths and the activity of insectivorous bats

THE lepidopteran family Arctiidae contains a number of species that emit high frequency sounds when tactually stimulated or exposed to the intense hunting cries of insectivorous bats¹⁻³. The sounds are produced as rapidly repeated clicks generated by the buckling action of the row of striae⁴ or microtymbals⁵ on the surface of the modified metepisternum (tymbal)⁶. The sounds seem to act as aposematic cues to discourage nocturnal, ultrasonically sensitive predators such as bats⁷. Flying bats will avoid otherwise palatable prey (mealworms) when accompanied by the recorded sounds of arctiid moths². If bats play an important role in the evolution of sound production in arctiids then bat activity levels in an area should influence the phenology of acoustically active arctiids there. By examining the incidence of some silent and sound-producing Nearctic arctiids and its relationship to ambient levels of bat activity, some of those influences can be demonstrated.

During the summers of 1975 and 1976, I collected moths from four ultraviolet lights located at the Queen's University Biology station near Chaffey's Locks, Leeds County, Ontario, Canada. The lights were inspected twice a night and any arctiids captured were immediately identified and tested for acoustic activity using a Brüel and Kjaer quarter-inch condenser microphone (type 4135) and measuring amplifier (type 2606) with a Tektronix 5103N storage oscilloscope⁷. Bat activity was monitored at the same location using an automated ultrasonic sensing system with detectors tuned to 40 kHz (ref. 8) directed over a lake where bats commonly fed. This location ruled out the possibility of detecting other sources of biological ultrasound (for example, insects).

Of the 24 arctiid species captured at the lights, 17 produced sound under light to heavy tactile stimulation (Table 1). Although the sounds generated by the moths were

extremely variable in amplitude-time patterning, frequency and intensity^{7,9} the degree of tactile stimulation required to elicit sound production was relatively constant within the categories listed in Table 1 for each species. A trend which soon became obvious was the timing of the emergence of silent and sound-producing species. During both summers, it was the silent species that appeared in the spring, and it was not until mid-July that a substantial proportion of moths caught at the lights were sound-producing species.

A comparison of the ambient bat activity levels in the area with the incidence of sound-producing arctiids (Fig. 1) reveals a close relationship between the two observations. A closer look at two genera lends insight into this relationship.

Phragmatobia (Stephens) includes two similar species that were captured at the study site: *P. assimilans* (Walker) and *P. rubicosa* (Harris). *P. assimilans* is a spring moth emerging early in May and disappearing before June. *P. rubicosa*, on the other hand, is a summer species, emerging in late July and remaining until August. *P. rubicosa* is an acoustically active species and will emit sounds under moderate tactile stimulation; its congeneric, *P. assimilans*, however, is silent. Scanning electron micrographs of the tymbal surfaces (Fig. 2) reveal that both species possess microtymbals but the band appears less defined in *P. assimilans* and may be vestigial.

The two species of the genus, *Hypoprepia* (Hübner), *H. fucosa* (Hübner) and *H. miniata* (Kirby) are also superficially similar in size and coloration¹⁰ and are also separated in their emergence times. *H. fucosa* appears from July to mid-August, whereas *H. miniata* appears somewhat later. Both species in this genus, however, emerge during periods of high bat activity and produce sound when lightly stimulated.

Animals exposed to unique forms of predation may develop predator-specific defences¹¹ that act directly on the particular characteristic of the relevant predator. Thus, the high frequency emissions of certain arctiids may be

Table 1 Acoustic activity of arctiids surveyed in chronological order of emergence

Species	n	Degree of stimulation required to elicit sound§	Time of emergence	
			Earliest date of collection	Latest date of collection
<i>P. assimilans</i> (Walker)	8*	Silent species	3/05/75	14/05/75
<i>Spilosoma virginica</i> (Fabricius)	20*	Silent species	13/05/75	22/07/75
			1/08/75	24/08/75‡
<i>S. prima</i> (Slosson)	4†	Silent species	13/05/75	05/06/75
<i>S. congrua</i> (Walker)	5†	Silent species	13/05/75	05/06/75
<i>Halysidota caryae</i> (Harris)	10*	Silent species	29/05/75	14/06/75
<i>Hyphantria cunea</i> (Drury)	15*	Silent species	10/06/75	18/06/75
<i>Callarctia virguncula</i> (Kirby)	3*	Heavy	15/06/75	22/06/75
<i>Pyrrharctia isabella</i> (Smith)	25*	Moderate	16/06/75	25/06/75
			01/08/75‡	
<i>Halysidota tessellaris</i> (Smith and Abbot)	35*	Moderate	15/06/75	30/07/75
<i>Cyenia tenera</i> (Hübner)	30*	Light	17/06/75	07/07/75
<i>Callarctia anna</i> (Grote)	5*	Heavy		15/06/75
<i>Euchaetias oregonensis</i> (Strech)	9†	Light	14/06/75	16/07/76
<i>Estigmene acrea</i> (Drury)	5*	Silent species		07/07/75
<i>Hypoprepia fucosa</i> (Hübner)	30*	Light	07/07/75	08/08/75
<i>Callarctia virgo</i> (Linne)	3†	Heavy	07/07/75	18/08/75
<i>Euchaetias egle</i> (Drury)	5†	Light	17/07/75	28/07/75
<i>Holomelina aurantiaca</i> (Hübner)	7†	Moderate	17/07/75	18/08/75
<i>Haploa confusa</i> (Lyman)	4†	Heavy		17/07/75
<i>Haploa contigua</i> (Walker)	3†	Heavy		17/07/75
<i>Phragmatobia rubicosa</i> (Harris)	6†	Moderate	26/07/75	03/08/75
<i>Hypoprepia miniata</i> (Kirby)	6†	Light	26/07/75	24/08/75
<i>Lycomorpha pholus</i> (Drury)	2†	Light		28/07/75
<i>Crambidia pallida</i> (Packard)	5†	Light		01/08/75
<i>Callarctia arge</i> (Drury)	2†	Heavy		03/08/75

*Both sexes were tested and showed the same acoustical activity.

†Both sexes were not tested.

‡Second brood.

§Degrees of stimulation similar to Blest¹: light involves merely touching the moth; moderate indicates the moth had to be shaken or prodded; and heavy indicates that very severe handling was required.

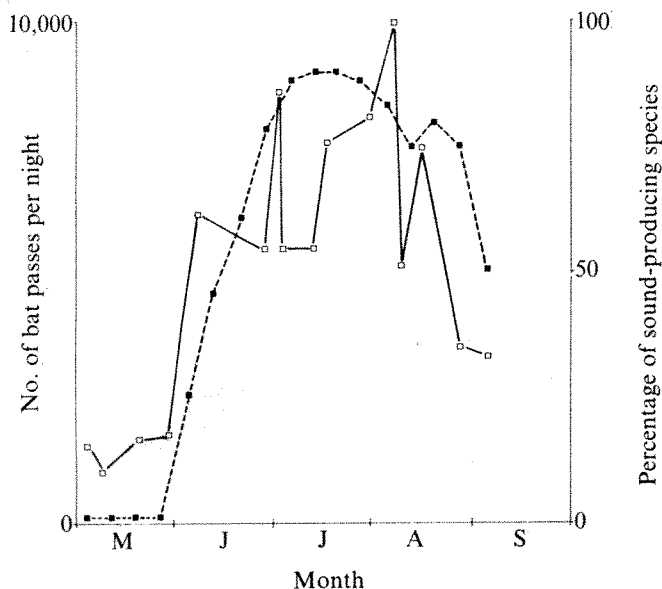
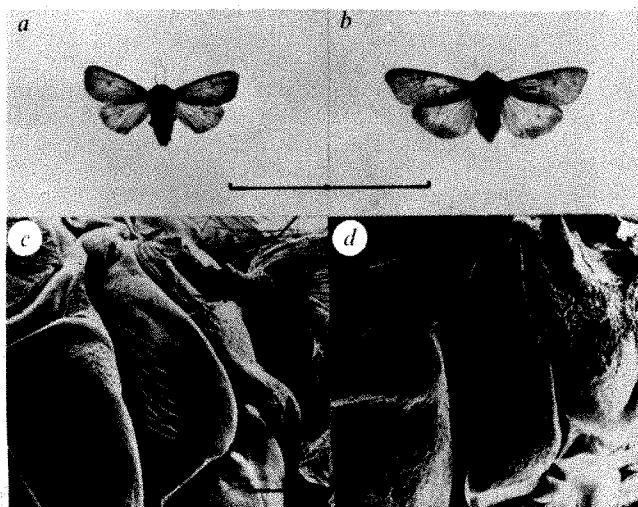


Fig. 1 Relationship between summer activity levels of insectivorous bats and phenology of sound-producing arctiid moth species. Bat activity (□) is recorded as 'bat passes'; incidence of acoustically active arctiids (■) are the averages per quarter month.

signals intended for the ultrasonically sensitive ears of predators such as bats. Since the maintenance of such specific defences should be dependent on the level of threat posed by the predators concerned, species exposed to lower levels of activity of these predators should not possess the defences of individuals more severely threatened. Therefore, the high proportion of silent arctiids present in the spring may be a response to the lower levels of bat activity at these times.

Phragmatobia offers an intriguing example of this relationship. *P. assimilans* is not only a silent species, similar to its acoustically active summer relative, *P. rubicosa*, but also possesses a tymbal structure which suggests that *P. assimilans* may have been a sound-producing species at one time. By shifting its emergence time to spring and thus avoiding the time of high bat activity (although this may not have been the original reason for the shift), *P. assimilans* no longer required a functional tymbal and now retains only a vestigial form of the organ. *Hypoprepia*

Fig. 2 a and c, Acoustically active summer arctiid, *P. rubicosa*, and its tymbal. b and d, Silent spring species, *P. assimilans*, and its non-functional tymbal with what appears to be a vestigial micro-tymbal band. Upper scale indicates 40 mm; lower scale indicates 1 mm.



illustrates a similar situation, the important difference being that both species emerge during high levels of bat activity and both produce sound.

Silent species that persist into or emerge in mid-summer (for example, *Spilosoma virginica* (Fabricius) and *Estigmene acrea* (Drury)) may be less vulnerable to bats for a variety of reasons. *E. acrea* is a very large arctiid and this may afford it sufficient protection from most hunting bats. Since arctiids will perform aerial evasive tactics in the presence of echo-locating bats¹², there may be a greater adeptness at this and other anti-bat defences that have allowed certain silent arctiids to exist in times of high bat activity.

As bats are not the only ultrasonically sensitive insectivorous predators that arctiids must contend with, it is likely that the ambient summer levels of non-Chiropterans (for example, *Peromyscus*) may also exert an influence on the phenology of sound-producing arctiid species. The diverse interactions that arctiids have with their predators are poorly understood and must be clarified before some of the puzzling problems associated with comprehending this unique form of defence can be dealt with.

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Survival value of fever in fish

THE phenomenon of behavioural fever, manifested as an increase in preferred temperature following injection of live or killed bacteria, or other pyrogenic substances, has been demonstrated in bony fishes¹⁻³, amphibians^{4,5}, reptiles⁶⁻¹⁰, and mammals^{11,12}. Ectothermic vertebrates, including some newborn mammals¹¹, are unable to increase their body temperatures much above ambient by physiological means, and so are largely limited to behavioural thermoregulation by selecting favourable temperatures in their environment. The behavioural febrile response of a lizard, *Dipsosaurus dorsalis*, has been shown to have adaptive value^{8,9-10} in conferring increased survival during infection by the Gram-negative bacterium *Aeromonas hydrophila*. *A. hydrophila* is pathogenic to bony fishes, amphibians and reptiles, causing haemorrhagic septicaemia. All three classes show similar (1-5 °C) elevations in preferred temperature in response to injection of this bacterium¹⁻¹⁰. The absolute preferred temperatures, however, both normal and febrile, differ considerably among these vertebrates. The preferred temperatures of lizards such as *D. dorsalis* (about 40 °C) are quickly lethal to most fishes and amphibians. We report here that fever significantly

enhances survival of goldfish, *Carassius auratus*, at febrile temperatures about 10 °C lower than those of *D. dorsalis*, after injection with live *A. hydrophila*.

To study the thermoregulatory behaviour of fish we used an electronic shuttlebox device which allows a fish to control water temperatures and, thereby, its body temperature, by its movements between chambers monitored by photocells^{13,14}. The behaviour of a fish in this device is equivalent to seeking out preferred temperatures in its natural environment¹³. At least some fishes have been shown to exhibit circadian changes in preferred temperature^{14,15}. Specifically, the goldfish exhibits a diel rhythm of preferred temperature with a pre-dawn peak (W. W. R., M. E. Casterlin, J. K. Matthey, S. T. Millington and A. C. Ostrowski, in preparation). Accordingly, for this study we pooled data for 24-h periods¹⁻⁴, to simplify presentation of the data by showing changes in mean preferred temperature for 24 h before and after injection with bacteria.

Earlier work with largemouth blackbass (*Micropterus salmoides*) and bluegill sunfish (*Lepomis macrochirus*) indicated a mean febrile increase in preferred temperature of 2.6 °C after injection with killed *A. hydrophila*. We therefore used a nominal febrile elevation of 2.5 °C to represent a typical fever in fish. We tested 10 goldfish in our thermoregulatory apparatus (Ichthyotron)¹³. These had a mean baseline (afebrile) preferred temperature of 27.9 °C, which agrees well with a previously published final preferendum value of 28.1 °C for *C. auratus*^{16,17}. Using a nominal 28 °C baseline temperature and the nominal 2.5 °C febrile elevation value, we tested three groups of 25 goldfish at fixed temperatures of 25.5 °C, 28.0 °C and 30.5 °C for 72 h. These represented, respectively, hypothermic, normothermic and febrile temperatures. The 75 goldfish, weighing about 4 g each, were injected intraperitoneally with 0.1 ml of sterile pyrogen-free fish saline containing 2×10^6 live *A. hydrophila* cells.

Goldfish maintained at a febrile temperature of 30.5 °C (Fig. 1) had the highest survival rate (84%), while only 64% survived at 28.0 °C and 24% at 25.5 °C. A control group of 10 fish, injected only with sterile saline, all survived at 25.5 °C. Another 10 fish injected with live *A. hydrophila* and allowed to thermoregulate in the shuttlebox attained an unexpectedly high mean febrile preferendum of 32.7 °C, a mean increase of 4.8 °C. The circadian rhythm of preferred temperature was maintained around the higher set-point. The high 4.8 °C febrile increase may have been due to multiplication of the live bacteria, resulting in a higher weight-specific dosage in these goldfish, which were smaller than the bluegills and bass tested previously¹ with killed bacteria. It may be relevant that the ultimate upper

incipient lethal temperature of goldfish is unusually high¹⁶ (about 41 °C) for fish—about 6 °C higher than that of bluegill and bass. None of the 10 febrile thermoregulating goldfish died, so the 32.7 °C temperature afforded even greater protection (Fig. 1) than did the nominal febrile temperature of 30.5 °C.

To examine the effect of temperature on growth rate *in vitro* of *A. hydrophila*, we grew cultures at 28, 30 and 33 °C (Fig. 2). We determined growth curves by inoculating 100 ml of glucose nutrient broth or brain-heart infusion broth with 0.1 ml of a 14 h broth culture; at 1–2 h intervals the cultures were plated and turbidometric readings made. There was no significant difference among the growth curves at the three test temperatures (Fig. 2), so the survival value of the fever apparently cannot be attributed to an effect of temperature on bacterial growth within the range of temperatures selected by goldfish. This is also true for lizards (*D. dorsalis*) at still higher temperatures⁵.

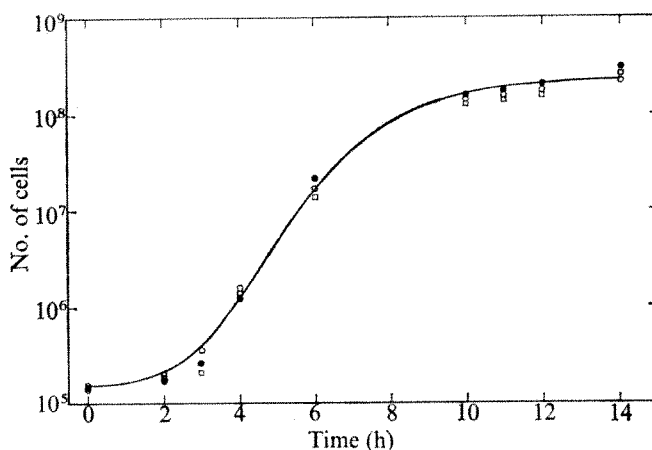


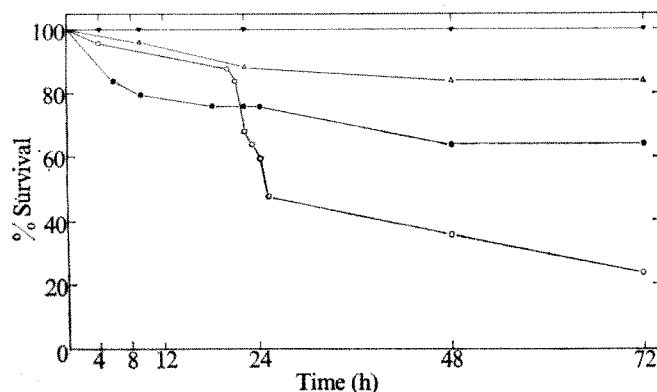
Fig. 2 Growth curves for three *A. hydrophila* cultures *in vitro* at 28 °C (□), 30 °C (○) and 33 °C (●).

Immunological responses of fishes and other poikilothermic animals have been shown to be significantly affected by temperature^{18,19}. The critical temperature levels are species-specific¹⁸, and vary considerably among classes¹⁹. For example, the normal body temperature of the lizard *D. dorsalis*⁵⁻¹⁰ exceeds even the febrile temperatures of the fish species studied, and indeed would be lethal to most fishes and amphibians. It thus seems that the absolute febrile temperature *per se* is less important than the febrile elevation above temperatures normal for the species.

Similar enhancement of survival during a viral disease by elevation of the water temperature has been reported for sockeye salmon, *Oncorhynchus nerka*²⁰. Mortality was prevented at 18 °C, which is 3.5 °C above the 14.5 °C normal preferred temperature of this cold-water salmonid species¹⁷. Amend²⁰ did not study behavioural responses to the virus infection. The virus was not destroyed by the 18 °C temperature, and enhancement of some critical hormone or enzyme activity was postulated as the cause of the increased survival²⁰. Another possibility is that increased phagocytosis could result from increased vascular permeability leading to greater mobility of the antigen-processing cells in the early stages of infection. It has also been suggested²¹ that there may be a relationship between temperature-dependent toxic effects of various substances and their effects on preferred temperatures of fishes, which might serve to reduce mortality.

Survival of infection can apparently be enhanced by fairly short-term increases in temperature, as the heliothermic *D. dorsalis* is able to thermoregulate only during the day, with body temperatures dropping to ambient

Fig. 1 Survival curves for goldfish at four temperatures after intraperitoneal injection with live *A. hydrophila*. Three groups of 25 fish each were held at fixed temperatures of 25.5 °C (○), 28.0 °C (●), and 30.5 °C (△); 10 fish (▼) were allowed to behaviorally thermoregulate at a mean temperature of 32.7 °C.



(12 °C) at night¹⁰; the daytime elevation of body temperature is apparently sufficient to enhance survival of infection⁸⁻¹⁰. Similarly, our febrile infected goldfish experienced no mortality following removal to holding tanks at room temperature (23 °C) after thermoregulating for several days at elevated temperatures. Avtalion¹⁸ has shown that after fish are allowed to develop a positive immunological memory at a high temperature, they are then able to produce antibodies even at low temperatures. It thus seems likely that some initial step in the immune response is enhanced by a febrile increase above the normal preferred temperature of a species, and that this is effective even if the febrile temperature is not constantly or indefinitely maintained.

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Soluble fragments from fungal cell walls elicit defence reactions in crayfish

ALTHOUGH most fungi that parasitise arthropods are relatively harmless, many can penetrate different types of cuticle¹. Thus active defence mechanisms presumably operate *in vivo*. In crayfish, for example, resistance to fungal penetration is associated with melanisation of the cell wall of the fungus by host phenols and phenoloxidase². This constitutes a system for recognising the invading organism at different levels, from the cuticle surface inwards³. Melanisation in insect blood, due to phenoloxidase activity, is also induced by the presence of cells or cell walls of fungi^{4,5}. Crayfish phenoloxidase in cuticle and blood is highly and specifically activated in contact with the purified hyphal cell walls of most fungi but not with other plant cell walls⁶. We have now found that soluble β -1,3-glucans from fungal cell walls activate the enzyme and may serve as specific elicitors of recognition in crayfish of invading fungal parasites.

Haemolymph was bled from the abdominal haemocoel of crayfish, *Astacus astacus*, using a hypodermic needle (0.8 mm diameter, 21 gauge). Because phenoloxidase is released by blood cells during clotting, cell free serum from pooled, clotted blood was used as a source of enzyme⁶. The clot was homogenised and centrifuged for 10 min at 3,000g. The phenoloxidase activity of the serum obtained was determined in a mixture of 0.2 ml serum, 0.1 ml dihydroxy-

phenyl alanine (dopa, 4 g l⁻¹), and 0.2 ml 0.01 M sodium acetate buffer, pH 5.2, with or without dissolved cell wall extract. The mixtures were incubated at room temperature, about 22 °C, in an open microscope slide cuvette⁷ for about 30 min. Before addition to the serum mixture the cell wall extract was preheated to 100 °C for 20 min to disperse aggregates. After covering the slide cuvette with a coverslip the activity of phenoloxidase on dopa was calculated from the increase in absorbance at 480 nm using a Shimadzu MPS-50 L photometer⁷.

Zymosan (purified cell walls from *Saccharomyces cerevisiae*) activated crayfish phenoloxidase, especially in regions close to the cell wall surface (Fig. 1). This effect is similar to that seen in fungal cell walls purified in alcoholic KOH⁸. Furthermore, the supernatant from centrifuged suspensions of Zymosan or purified cell walls (even after six washings) of different fungi strongly activated phenoloxidase. Apparently, water-soluble fragments with activating capacity were released in very small concentrations from the solid cell walls.

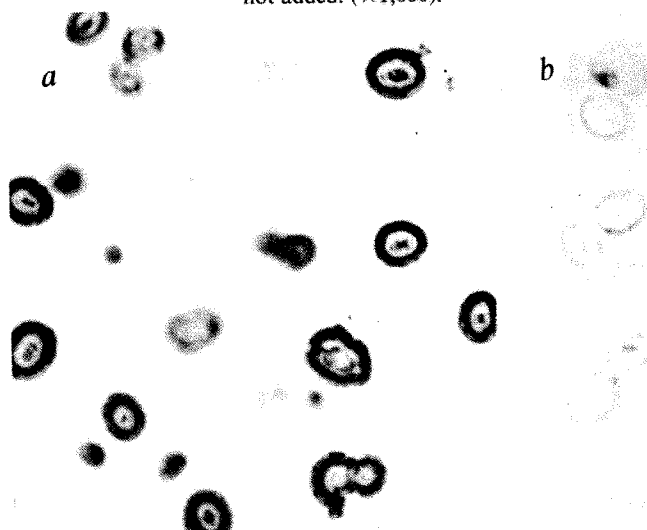
In the present work we used the supernatant (containing the yeast cell wall activator) from a 1% suspension of Zymosan suspended in 0.01 M sodium acetate, pH 5.2. The molecular weight of the activator was estimated on a column (26 mm $\times$ 550 mm) of Sephadex G 200, using acetate buffer at pH 5.2. Blue dextran 2000 (Pharmacia), bovine γ globulin and trypsin (both Sigma) were used as standards. When compared with the standards, the apparent molecular weight of the activator was between 10⁵ and 10⁶; small amounts of material with a molecular weight between 3 $\times$ 10⁴ and 10⁵ were also present. It seems that the elicitor is not a distinct molecule but consists of macromolecules or molecular aggregates of varying size.

Most elicitor activity was not bound to a cation (SP-Sephadex C 25), nor to an anion exchange resin (DEAE-Sephadex A 25) when the material was dissolved in and eluted with 0.01 M sodium acetate buffer, pH 5.2, and 0.01 M Tris-HCl buffer, pH 8.2, respectively and was apparently not charged to any detectable extent at these pH values.

The activator solution (from 1% Zymosan) contained about 0.4 g of dry material per l (desiccation at 100 °C for 3 h). It activated phenoloxidase even when diluted 10,000-fold. Elicitor activity could therefore be measured at least down to a concentration of about 10⁻¹¹ M.

The yeast cell wall elicitor was also pretreated at 22 °C for 1-24 h with different hydrolytic enzymes (0.2 mg ml⁻¹) to investigate the possibility that the elicitor molecules

Fig. 1 Zymosan activation of phenoloxidase of the cell free serum of crayfish. In the presence of a substrate, dopa, the cell walls thereby become melanised (dark). a, Dopa added; b, dopa not added. ($\times$ 1,000).



could be inactivated (Table 1). The samples were heated (100 °C, 20 min) to destroy lytic enzyme activity and disperse aggregates before addition to the test mixture containing serum and dopa. Results were compared with the corresponding heated control sample containing the hydrolytic enzyme alone. As *Saccharomyces* cell walls contain mostly β -1,3-glucans⁸, it was not surprising that endo- β -1,3-glucanase affected the soluble cell wall fragments. This enzyme increased the activating capacity of the cell wall fragments (Table 1), especially after short exposure. Exposure for several days, however, did not destroy the activator substance or significantly decrease its molecular weight. Owing to the enzyme activity the number of active groups on the glucans was probably increased by exposing new, activating groups derived from the splitting of the large molecules. Digestion of the soluble fraction from 1% Zymosan suspension for 0.5 and 3 h with endo-glucanase followed by vacuum dialysis showed that none of the active molecules formed by hydrolysis had a molecular weight less than 20,000.

After long treatment with endoglucanase (Table 1) the additional, activating groups, but only these, were apparently digested and destroyed by the enzyme. Exo- β -1,3-glucanase, however, almost completely destroyed all the activating capacity of the glucans, suggesting that chains of β -1,3-linked glucose residues are involved.

Proteases had little or no effect and did not change the molecular weight of the bulk of the activator. Treatment with cellulase increased the activating effect considerably (Table 1). Traces of β -1,3-glucanase activity may be responsible for this, unless cellulosic components were also present in the glucans.

Since β -1,3-glucans seem to be involved in the activation, laminaran, a sparsely branched β -1,3-, β -1,6-glucan (from the alga *Laminaria hyperborea*) with an overall degree of polymerisation of about 30 glucose units, was also tested against the crayfish serum. At concentrations of 4×10^{-4} to 2×10^{-3} M this compound strongly inhibited phenoloxidase activity but at 4×10^{-6} to 10^{-6} M it was stimulatory. Activation could be detected down to about 10^{-7} M. The reason for the inhibitory effect at the higher concentrations is not yet known.

Pretreatment of 0.1% laminaran for 24 h with endo- or exo- β -1,3-glucanase destroyed activating capacity (Table 1).

Table 1 Effect of soluble *Saccharomyces* cell wall fragments and of laminaran on crayfish phenoloxidase after pretreatment with lytic enzymes

Pretreatment	Increase in activity of phenoloxidase*	
	Zymosan (%)	Laminaran (%)
None	600 (0.66)†	160 (0.18)
Pronase	600 (0.66)	—
Trypsin	650 (0.72)	—
Cellulase	900 (0.99)	—
Endo- β -1,3-glucanase	800‡ (0.88)	0 (0)
Exo- β -1,3-glucanase	60 (0.07)	0 (0)

Pretreatment of 1 mg ml⁻¹ laminaran (Koch-Light) and of the soluble fraction (0.4 mg substance per ml) from a 1% suspension of purified *S. cerevisiae* cell walls (Zymosan, Koch-Light) was carried out at pH 5.2 in 0.01 M sodium acetate buffer for 24 h. Lytic enzymes used (0.2 mg ml⁻¹) were: Pronase (Sigma), trypsin (Difco), cellulase (S173, from *Trichoderma viride*, having traces of β -1,3-glucanase activity) endo- β -1,3-glucanase (S176G, from *Rhizopus arrhizus*) and exo- β -1,3-glucanase (highly purified). Phenoloxidase activity against dopa was measured photometrically. Percentage increase in activity after addition of tenfold diluted, activating cell wall material or pretreated cell wall material is given.

*Measured after 30 min incubation. Phenoloxidase activity in absence of activating cell wall glucans was equivalent to A_{480} of 0.11 in the assay system.

†Figures in parentheses are A_{480} values in assay.

‡After 30 min pretreatment, 1,000% increase. After 3–5 d, increase was about 600%.

Thin-layer chromatography of the enzyme digests on silica gel (*n*-butanol/ethanol/water: 5/3/2) showed complete degradation of the glucan into mono- and disaccharides after 3 h of incubation with the enzyme.

Cell walls from many other fungi also activate the phenoloxidase from crayfish⁹. We treated activator-containing extracts from cell walls of *Polyporus annosus*, a basidiomycete, and *Aphanomyces astaci*, an oomycete, with the exo-glucanase as above. In both cases the activating capacity was destroyed indicating that the elicitor is a β -1,3-glucan and may be a general characteristic of most fungi.

Among vertebrate parasites fungi are less common than bacteria. The reason for this is not known and the vertebrate immune system cannot fully explain the discrepancy⁹. It is possible that soluble and chemically related cell wall fragments from different fungi, such as the elicitors described here, are also involved in the nonspecific recognition of fungi by vertebrates. It is interesting that a fungal β -1,3-glucan has been claimed specifically to induce anti-tumour activity in the mouse¹⁰.

Albersheim *et al.*^{11,12} have characterised fungal cell wall fragments which elicit host response in bean tissues, and the similarity with the fungal elicitor active in crayfish is striking. It is therefore tempting to speculate that most fungi cannot avoid producing 'warning signals' which are similar in different fungal groups and recognised by both plants and animals.

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Action of juvenile hormone mimics on the regulation of larval-adult and alary polymorphism in aphids

It has often been suggested that the development of the apterous and alate morphs of aphids is controlled by the corpus allatum (CA) and is therefore regulated by the same endocrine system that controls larval-adult development in monomorphic insects^{1–6}. Research on other polymorphic species, including the honey bee and termites, has shown that this function is compatible with the cyclical production of juvenile hormone (JH) during larval ecdyses as the morph-determining action of the hormone may be confined to a certain strictly defined critical period or may depend on specific hormone concentrations in the blood^{7,8}. In aphids the prenatal determination of late embryos as presumptive apterae has been attributed to high JH titres in the maternal haemolymph^{4–6}; and the subsequent realisation of the morph characters has also been thought to

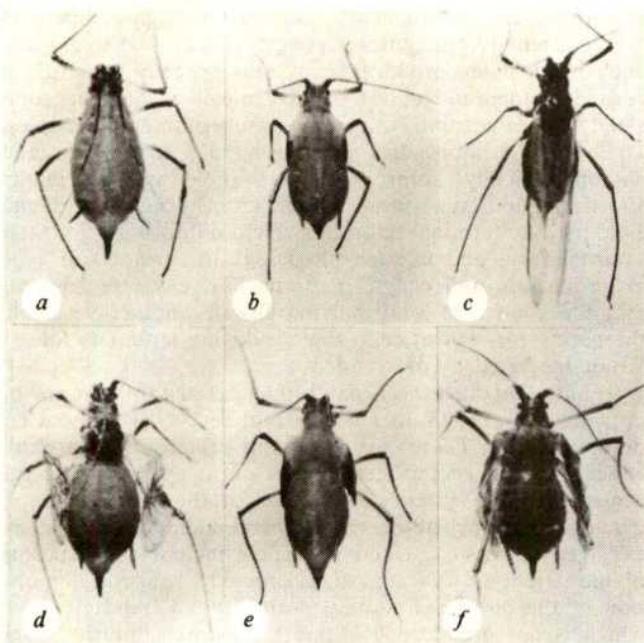


Fig. 1 *Megoura viciae*: a, normal adult aptera; b, normal alata in fourth (last) larval instar; c, normal adult alata; d, fifth-instar larval-adult intermediate produced by topical application of 10^{-3} μ g ZR-512 to a third-instar alate larva. This individual did not undergo a further ecdysis. e, Fifth-instar supernumerary alate larva showing maximum juvenilising effect. 10^{-2} μ g ZR-512 was applied to the third-instar alata. f, Sixth-instar larval-adult intermediate resulting from the ecdysis of a high grade supernumerary larva (as in e), produced by treating a third-instar alata with 10^{-2} μ g ZR-512.

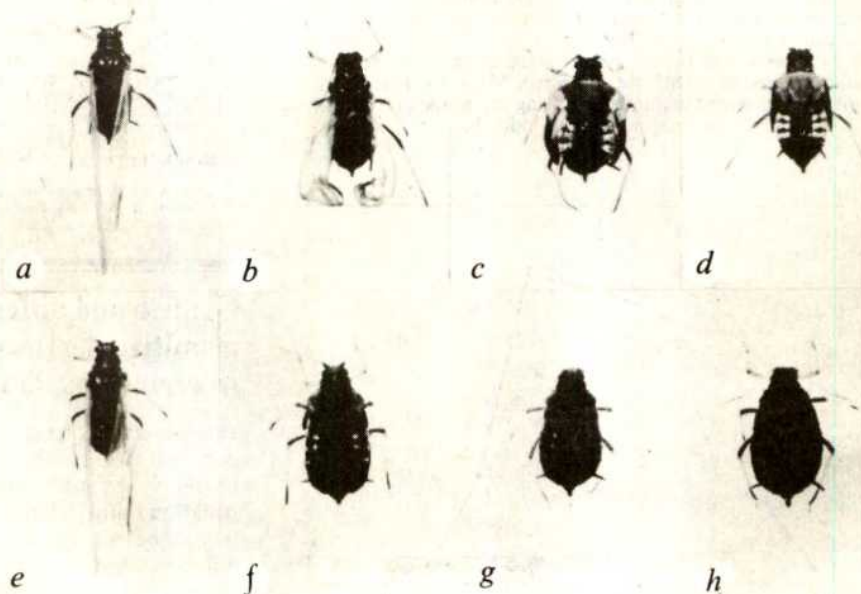
involve enhanced CA activity in the early instars of the developing larva^{2,3}. The evidence, which has been based on measurements of CA volume and on the effects of topically applied JH is nevertheless conflicting^{9,10}. The results reported here show that the intermediate and terminal forms produced by the application of juvenoids to developing alate aphids are unlike those resulting from authentic

apterisation initiated either by the absence of crowding or by exposure to long photoperiods. This does not support the persuasive argument that the possession of certain apparently juvenile characters by the 'neotenic' apterae (for example, the wingless condition of the adult) is a reliable indicator of JH involvement.

In early experiments with the vetch and bean aphid *Megoura viciae*, cecropia extracts with relatively slight JH activity were applied topically to alateform larvae in their penultimate (third) larval instar^{2,3}. After an externally normal ecdysis to the fourth instar, these individuals developed into adults with stunted wings, a reduced pterothorax and an abdominal pigment pattern resembling the adult aptera rather than the adult alata (Fig. 1a, c and d). The use of more potent synthetic JH mimics, however, has shown that this effect does not represent progress towards the apterous morph. After treatment with a saturating dose of JH mimic third instars develop into a supernumerary fifth-instar alate larva with unmistakable wing pads and a completely unsclerotised pterothorax (Fig. 1e). In addition, the genital opening remains closed and the ocelli are often suppressed. A further ecdysis follows. The few sixth-instar aphids that can extricate themselves from the old cuticle are often still somewhat juvenile in appearance (Fig. 1f). These more extreme features, which have been observed in several species^{5,11}, indicate that the juvenilising effect is similar to that in many other insects¹². It is not surprising therefore that other aphid morphs including the aptera and the wingless ovipara respond in a comparable manner^{13,14}.

It is less certain, however, whether juvenoids also affect morph determination, because this event occurs much earlier. In *Megoura*, for example, the morph type is controlled maternally just before birth¹⁵. To test this point, 1.0- μ g quantities of the juvenoids ethyl 3,7,11-trimethyl-dodecadienoate (ZR-512) (Zoecon), methyl 10,11-epoxy-farnesoate (C16-JH) (Eco-Control) or the Law-Williams (L-W) chlorinated farnesoate mixture¹⁶ were applied topically to the abdomen of parent apterae prepared as alata-producers by crowding them together¹⁷. Although all these growth regulators produce a maximum morphological response in the third-instar alata at a dose of only 10^{-2} – 10^{-3} μ g, the treated adults continued to produce alate

Fig. 2 *Aphis fabae*: a-d, range of forms produced by topical application of a JH mimic (Law-Williams) to second and third-instar gynoparae. Examples of four of the five arbitrary response categories are illustrated. a, Normal adult gynopara, untreated: 0% morphological effect (ME). b, Wings curled: 25% ME. c, Wings intermediate, pterothorax weakly sclerotised, large wax patches present: 75% ME. d, Maximal effect. A fifth-instar supernumerary larva with large larval wing pads and conspicuous wax patches: 100% ME. e-h, Range of forms produced by exposing presumptive gynoparae reared in short days to two long photoperiods during the first 2 d of development. The five response categories differ from the above. Examples of four are shown. e, Normal gynopara: 0% ME. f, Intermediate adult with small wing pads: 50% ME. g, Intermediate adult with some thoracic sclerotisation but no wing rudiments: 75% ME. h, Adult showing complete apterisation: 100% ME. Aphids placed in the 50% ME category in the JH series (not shown) have crumpled wings, partially obliterated sutures between the thoracic sclerites and small wax patches. Those assessed at 25% ME in the long day series have half-expanded wings but are otherwise normal alates.

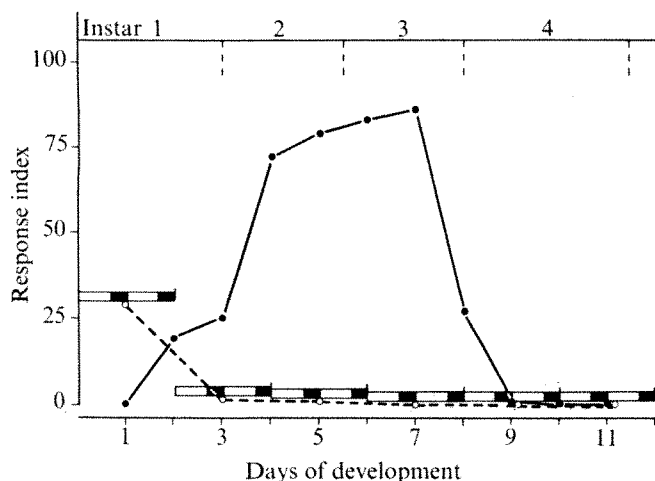


progeny when subsequently isolated on bean seedlings (*Vicia faba*). Apterous-producers, isolated before the final moult, were also unaffected by JH treatments and yielded only apterae. In another experiment, the 1-d-old progeny of crowded parents were sprayed with an acetone solution containing 0.5 µg of L-W mixture (sufficient to ensure a maximum response in groups of third-instar alateform larvae). The proportion of insects developing as apterae was not increased. There was therefore no indication that these JH mimics either influenced the morph-determining process in the parent or modified the alate character of the neonatal progeny immediately after determination.

The winged gynoparae of a host-alternating species such as the black bean aphid *Aphis fabae* have two advantages as experimental subjects. First, it is certain that every first-instar larva is a presumptive alata, provided the parent has developed in short days (12 h light: 12 h dark) at 15 °C. Second, the alate condition of the first-instar gynoparae is partially or completely suppressed by exposure to a threshold number of long days (16 h light: 8 h dark) during the first and second instars. The intermorphs and extreme forms can then be compared with the intermediates produced by JH treatments.

The range of morphological effects produced by JH is shown in Fig. 2. As in *Megoura*, the extreme forms are near-perfect fifth-instar supernumerary alate larvae with large wing pads, an unsclerotised pterothorax, a closed genital canal and, in this species, conspicuous larval wax patches (Fig. 2d). These juveniles invariably undergo an additional ecdysis. An assay based on these and other characters has been used in assessing the morphological effect (ME) of a JH mimic (0.5 µg L-W in acetone) when applied as an atomised spray to developing colonies of gynoparae, each comprising about 100 individuals. Figure 3 shows that the presumptive gynoparae display relatively little sensitivity in the first day or two of post-natal development, the single application of mimic again producing its greatest effect in the third instar (day 7). In contrast, exposure to two consecutive long photoperiods is maximally effective during the first 2 d of development (Fig. 3). As a result of this early treatment 67% of the presumptive gynoparae continued to develop as normal alate morphs (Fig. 2e), 8% developed as intermediates, some with small wing pads (Fig. 2f), while 25% developed as authentic apterae

Fig. 3 Time of responsiveness to treatments influencing juvenile-adult and apterous-alate characters in gynoparae of *Aphis fabae*. —, Response to a single application of L-W juvenoid when applied at constant dose on different days of larval development. — — —, Response to two long days applied on the days indicated by the photoperiod bars. The response index is calculated as $\Sigma (ME \times n)/N$, where ME is the percentage morphological effect, n the number showing this grade of response and N the total number of individuals.



without wing vestige or thoracic sclerotisation (Fig. 2h). Moreover, the intermediates and apterae gave birth to parthenogenetic daughters (virginoparae), whereas the unchanged alatae produced oviparous progeny as would a normal gynopara. Neither the intermediates nor the completely apterised aphids underwent supernumerary ecdyses. These results show that juvenilising JH treatments and photoperiodically controlled apterisation are maximally effective at different times during larval development and give rise to terminal morphs which differ both in their external form and in such physiological attributes as type of germarium and ecdysis pattern. This evidence suggests that the control of wing polymorphism, whether vested in the parent (*Megoura*) or in the developing larva (*A. fabae*), is not mediated by JH.

Aphids that have been determined as alata-producers by crowding give birth to a small number of progeny when decapitated^{18,3}. These are invariably apterous. The same effect can also be produced in *Megoura* by sectioning the central nervous system at the level of the suboesophageal ganglion and by other treatments which would not be expected to affect CA activity. These include the exposure of the parent to CO₂ anaesthesia for 30 s, and the amputation of the distal extremities of the legs. The latter effect, which is not produced by other comparable injuries, such as antennal amputation, seems to be correlated with the removal of certain whip-like trichoid sensilla adjacent to the eversible tibial climbing organ. Evidently, the loss of the normal sensory input to the thoracic ganglion both releases the parturition reflex from inhibition and elicits the morph change in the fully developed embryo. These considerations suggest that the effector is neural, but there is as yet no evidence that the control mechanism involves a blood-borne hormone.

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Growth and differentiation of a primitive nervous cell line after *in vivo* transplantation into syngeneic mice

In vitro study of nerve cell differentiation has been primarily concentrated on the examination of cell lines that, at least partially, express terminal steps of differentiation. The isolation and characterisation of precursor populations would be an aid to further understanding of nerve cell differentiation. I present here evidence for a clonal line of such a nerve cell precursor type which, after *in vivo* passage, is able to give distinct neuronal phenotypes. These phenotypes are potentially lineage related, and show qualitative

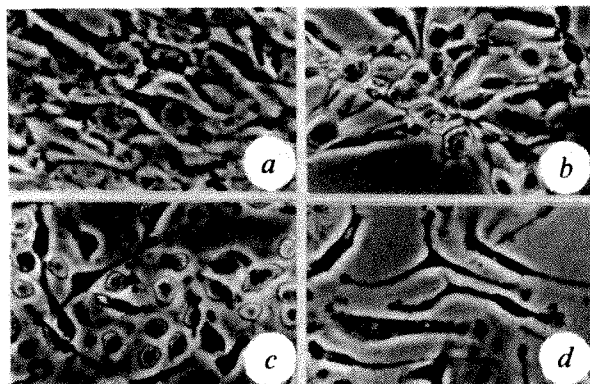


Fig. 1 Diversity of tumour clones. Phase contrast photographs of four hypothalamic clones issued from a tumour of the primitive F7 cells. *a*, A primitive tumour clone. *b*, A newly differentiated cell type (4-3). *c* and *d*, Two neurosecretory cell types as compared to C7 clone's morphology¹. Tumour was sterily excised, finely minced with scissors and then mechanically disrupted with a Pasteur pipette. Cells and small aggregates were plated in 30 ml Falcon plastic culture bottles containing 2-3 ml of Ham F10 medium GIBCO supplemented with 15% heat inactivated horse serum, 2.5% foetal calf serum, 1.5% of 200 mM L glutamine, penicillin (50 IU ml⁻¹) and streptomycin (50 µg ml⁻¹). Cells were grown in an atmosphere of 10% CO₂ 90% air.

differences in the expression of their hypothalamic neuronal function.

Clonal cell line C7 isolated from primary cultures of 14-d-old mouse hypothalamus embryo (A/J) after SV 40 transformation initially retained the capacity to form neurophysin and vasopressin¹, a function unique to some hypothalamic neurones *in vivo*. After several passages however, some cells lost the capacity to synthesise neurophysin and vasopressin. Subclones of the original C7 line were isolated and morphologically, histochemically and immunologically characterised. The primitive nature of one of these clones, F7, derived from such neurosecretory cells, has been tentatively established on the basis of its ultrastructural features and its negative response to all of the tests that were used to identify neurosecretory cells².

If it were possible to demonstrate that a reversible switch from this primitive phenotype F7 back to the differentiated one had occurred, it would give a means of determining whether F7 is a precursor cell antecedent to a definitive neurone; although such a reversible switch has not been observed *in vitro*, this differentiation could be initiated by passage of these primitive nerves through syngeneic mice. The proliferating cells originating from these primitive nerve cells, after transplantation in mice, give rise to tumour cell populations containing both primitive and differentiated cells, as defined by the presence of immunoreactive neurophysin and β -glucuronidase activity.

The primitive nerve cell clone F7 (10⁷ cells) was injected subcutaneously into male A/J mice. Tumours arose after a latent period of 5-10 d. The ability of such transformed cells to proliferate was surprising in view of the fact that the original C7 neuronal type was unable to produce tumours in the same conditions. These tumours were excised and cultivated. Primary cultures were maintained for about 6 weeks in the same flask before cloning and medium was changed by half every 2-4 d. Cells originally plated grew into three distinct morphological types (Fig. 1*a-d*) among which one type was predominant (type 1*b* in Fig. 1). Colonies of these three distinct phenotypes were dissected out with a 10 µl Pedersen micropipette, scraping only the centre of the colony to get clean cell populations.

The first resulting homogeneous population of cells grew in a non-overlapping monolayer, with a division time of 12-16 h (Fig. 1*a*). The model chromosome number of the cells was the same as for the original F7 clone (70

chromosomes). This population grew as a stable line. The Gomori's paraldehyde fuchsin and β -glucuronidase histochemical reactions used before to identify the neurosecretory cells were consistently negative on these primitive cells². The immunocytochemical detection of neurophysin using bovine neurophysin I and II antisera also gave negative results. These cells closely resembled the originally inoculated F7 cells.

The second class of cells were very refractile and interconnected by long narrow processes; they had a division time of about 24 h (Fig. 1*b*). These cells were positive for the two histochemical markers of neurosecretory cells: the Gomori's paraldehyde fuchsin and β -glucuronidase reactions (Table 1). They gave a positive immunocytochemical reaction for neurophysin using bovine neurophysin I antiserum (Fig. 2). They gave a negative reaction using bovine

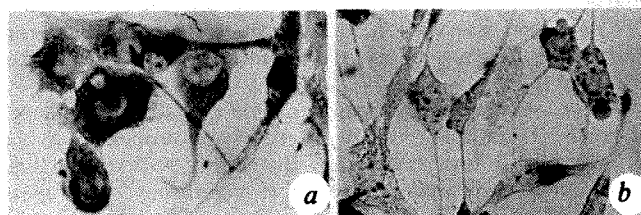


Fig. 2 Immunocytochemical staining with Ab-NI (1/50). *a*, A newly differentiated tumour cell clone (4-3). *b*, A primitive tumour cell clone (PT). ($\times 300$.)

neurophysin II and vasopressin antisera, however (Table 1). These cells were unstable in the culture conditions—4 to 5 months after the excision of the tumour corresponding to about 17-20 subcultures, the level of intracellular neurophysin began to decline as indicated by the immunocytochemical detection. At the same time, these cells started to divide faster, and progressively took the phenotype of the originally fast dividing inoculated F7 cells.

A third type of cell could be identified among the heterogeneous population derived from the tumour: a neuronal refractile cell type (Fig. 1*c* and *d*) closely resembling the

Table 1 Results of the cytochemical and immunocytochemical staining of the primitive tumour cell type and the newly differentiated tumour cell type, as compared to the original clones (C7 and F7)

Clone	Gomori's paraldehyde fuchsin	β -glu- cunonidase	Ab N I 1 : 50	Ab N II 1 : 10	Ab LVP 1 : 10
Tumour Primitive type (PT)	—	—	—	—	—
Differentiated type (4-3)	++	++	++	—	—
Control neuro- secretory clone (C7)	+++	+++	+++	+++	+++
Primitive clone (F7)	—	—	—	—	—

Gomori's paraldehyde fuchsin staining and cytochemical detection of β -glucuronidase were as previously described². Neurophysin and vasopressin were localised using the peroxidase-labelled antibody technique with antibodies against bovine neurophysin II (Ab-N II), bovine neurophysin I (Ab-N I) and Lys-vasopressin (Ab-LVP). Cells grown in plastic dishes were treated according to Tougaard *et al.*⁷. The specificity of the reaction was tested by using normal rabbit serum. Rabbit antiserum to bovine neurophysin II was specific for neurophysin II and cross-reacted very poorly with bovine neurophysin I⁸. A dilution of 1/10 was used. Rabbit antiserum to bovine neurophysin I cross-reacted slightly with N II (ref. 9). A 1/50 dilution was used. Anti-lys-vasopressin serum was obtained in rabbits immunised with vasopressin coupled to charcoal. A 1/10 dilution was used.

neurophysin-vasopressin synthesising C7 clone¹. These cells grew in a dispersed state, and the number of colonies of such type was small as compared to the other types. Their division time was about 2–3 d. They were rapidly overgrown by other much faster dividing type of cells and they could not be dissected or subcultured. On the basis of their morphological analogy with the other neurophysin-vasopressin clones already isolated², and their slow growth rate as compared to the fast growth rate of the F7 type clones, they may represent the neurophysin-vasopressin synthesising cell class.

In summary the primitive nerve cell line F7, when injected subcutaneously into syngeneic mice, gives rise to tumours which after subculture contain not only primitive cells but also a new type of differentiated cells. These newly differentiated cells can be distinguished from primitive cells on the basis of their morphological and growth rate alterations, their neurophysin content, and their positive reaction towards β -glucuronidase activity, a neurosecretory cell marker *in vitro*³. These cells which contain neurophysin but are negative with a vasopressin antiserum may correspond to a step of differentiation towards the neurosecretory type.

These observations support the view that cells undergo stable modifications in their differentiated properties in the *in vitro* conditions, but retain the ability to express their specific function under favourable conditions such as *in vivo*. It seems unlikely that the changes observed in the populations could be due to selective growth of pre-existing neurophysin cell type, since the F7 cell population inoculated into the animal originated from a single cell progeny, and grows as a stable line. Its growth rate being about four times faster than the neurophysin-vasopressin synthesising cell type, it has a selective advantage in the culture conditions, and would rapidly overgrow a neuronal cell, if any. If F7 cells were able to segregate neurophysin synthesising cells *in vitro*—it could not be observed with our markers—this would argue for F7 being a precursor cell type of a neurophysin cell. Finally inoculation of 10⁷ cells of the neurophysin-vasopressin synthesising C7 clone¹ into syngeneic mice did not give any tumour.

The *de novo* synthesis of a differentiation gene product associated with the *in vivo* passage is a striking phenomenon. Such induced morphological and cell function alterations have been reported in the case of endocrine tumour cells: hormonal activity may be restored in tumour cells which no longer synthesise their specific product, by animal passage³. In the case of teratocarcinoma, lines can be established that remain undifferentiated. Some of these, when injected into mice, give rise to tumours containing various tissues^{4–6}. In our case more studies are needed to determine whether this primitive cell F7 is only a precursor of a neurosecretory cell lineage or is a more remote stem cell for nerve cell types such as glial cells or other types of neurones. But these cells might be used as a suitable model to study the sequence of steps involved in the progressive differentiation of a neuronal lineage.

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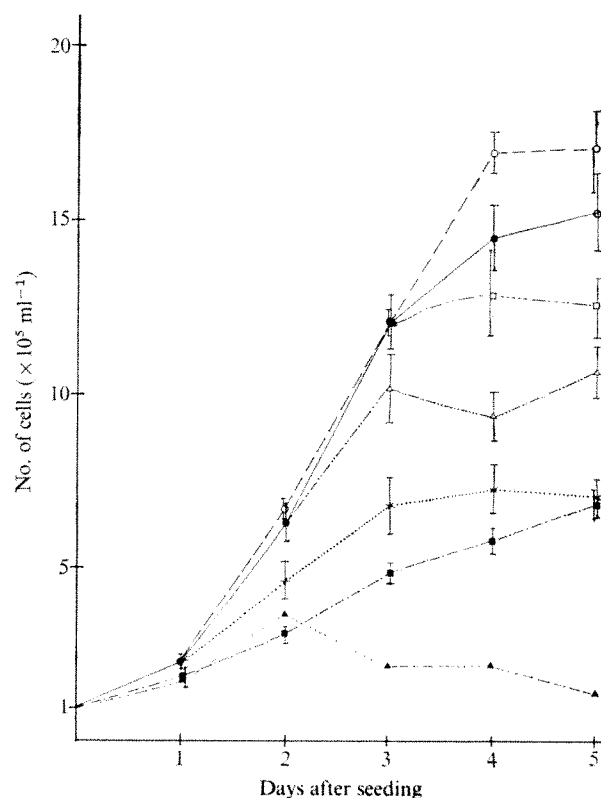
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Interferon inhibits dimethyl sulphoxide-induced erythroid differentiation of Friend leukaemia cells

INTERFERON (IF), as well as showing antiviral activity is able to inhibit cell growth and metabolism both *in vivo*^{1,2} and *in vitro*^{3–5}. In the *in vitro* systems used, dead cell counts are always very low, indicating that IF inhibits cell growth by decreasing their doubling potential^{4,5} without appreciable cell death. Since we have shown previously that IF inhibits *in vivo* growth of Friend virus (FLV)-induced murine erythroleukaemic cells (FLC)⁶, we have studied the *in vitro* effects of IF addition to cultures of FLC. These cultures, on exposure to dimethyl sulphoxide (DMSO), undergo massive erythroid differentiation accompanied by haemoglobin (Hb) synthesis^{7,8} as a consequence of production of globin messenger RNA^{9,10}. Here we present evidence for a dose-dependent and reversible inhibitory effect of IF on both growth and erythroid differentiation of DMSO-stimulated FLC.

Several preparations of interferon were used in the

Fig. 1 Growth curves of Friend leukaemia cells (FLC) treated with DMSO and/or IF. FLC (clone 745A from C. Friend), grown in suspension cultures in Dulbecco's modified Eagle's medium, supplemented with 15% foetal calf serum and antibiotics, kept at 37 °C, were seeded at 10⁵ cells per ml with no further treatment (○); with 1.5% (by vol.) DMSO (●) and with 1,000 units of IF (■). To samples of 1.5% DMSO-treated cultures, 1,000 U of IF were added either on day zero (▲), or on day 1 (×), or on day 2 (△), or on day 3 (□). Data shown are means $\pm$ s.e.m. of viable (trypan blue dye-exclusion method) cell counts from several experiments. Mouse IF preparations were from Drs I. Gresser and F. Dianzani. Procedures for IF production, partial purification and titration have been described¹⁶.



present study: IF dosages are given in mouse interferon units equal to 4 mouse interferon reference standard units.

IF was initially added at the time of cell seeding (day zero) without DMSO. A pilot experiment showed that IF at 1,000 units per ml per 10^5 cells inhibited FLC growth five-fold with no cell death, as judged by trypan-blue dye exclusion method (Fig. 1, ■). This inhibition was markedly enhanced when IF and DMSO were given simultaneously on day zero. In these conditions, where not even one round of cell doubling occurred and a few dead cells were observed on days 4 and 5, no Hb synthesis was detectable. This is in keeping with reports^{11,12} that at least one cell doubling is required for DMSO induction of Hb synthesis, although this is not necessarily true for other inducers such as butyric acid¹³.

When, instead, IF was added 24 h later (day one) to cultures seeded with DMSO so that the cell population was allowed to undergo at least one doubling before being exposed to IF (Fig. 1, ×), the addition of IF still inhibited cell growth. The IF-treated cultures reached on day 5 a concentration of about 7×10^5 per ml without appreciable mortality. This implies that the cells doubled almost twice after the addition of IF. In these conditions, used in most experiments described hereafter, a ninefold reduction of Hb production, as measured on the fifth day of culture, was observed (Table 1). Hb values of DMSO+IF-treated FLC remained low even when tested on day 7. This effect was much less evident when IF was added on day 2 or 3. On the other hand, there was only a twofold reduction in the accumulation of ⁵⁹Fe and its incorporation into haem.

The IF effects on cell replication and differentiation are dose-dependent (Fig. 2). It is noteworthy that at least 500 units of IF per ml per 10^5 cells are needed to appreciably detect them. This most probably accounts for the apparent discrepancy between our data and those of Swetly and Ostertag¹⁴ and Lieberman *et al.*¹⁵, who found that doses of IF capable of inhibiting FLV production by FLC *in vitro* had no appreciable effect on cell growth and DMSO-induced erythroid differentiation of these cells.

That the observed effects are attributable to IF itself rather than to unknown contaminants of IF preparations is indicated by the following evidence. First, effects induced by IF preparations of various degrees of purification ($\geq 10^6$ units per mg protein for Gresser preparations, 10^5 or 10^4 units per mg protein for Dianzani preparations) are indistinguishable. Second, mouse IF proved equally active regardless of the cell type used as source of IF (brain, fibroblasts, ascites cells) and of the various inducers (West Nile virus or Newcastle disease virus). And third, rabbit heterologous IF or mouse mock IF (medium from uninduced cultures) were ineffective (data not shown).

Inhibition of growth and differentiation of FLC can be fully reversed by thorough washing to remove IF (Table 2). Growth curves of IF-treated cell cultures, following washing

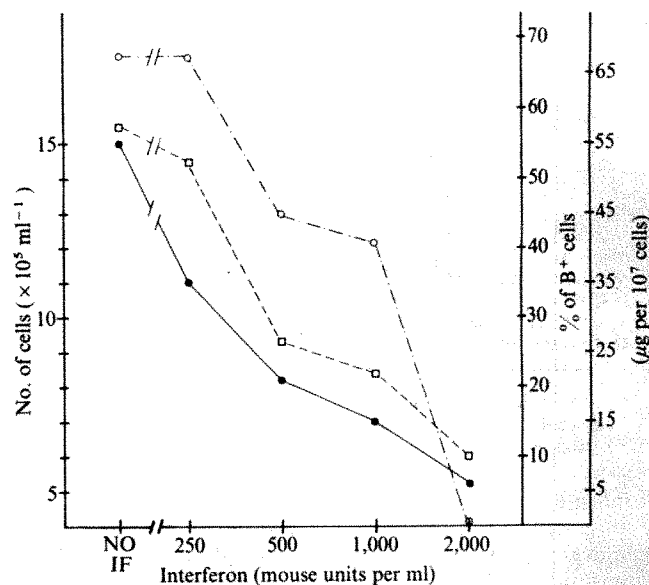


Fig. 2 Dose response curves of IF inhibitory effect on FLC growth and DMSO-induced differentiation. Data are the average of triplicate samples of 5-d cultures seeded with 1.5% DMSO and given the indicated IF amounts on day 1. Benzidine-positive (B+) cells counted on cyto-centrifuge sediments stained with Ralph's technique. At least 500 cells were scored. Viable cell counts as for Fig. 1. Haemoglobin measurements were carried out on cell lysates as described for Table 1. ●, No. of cells per ml. □, % B+ cells. ○, $\mu\text{g Hb per } 10^7 \text{ cells}$.

and reseeded at 10^5 cells per ml are indistinguishable from controls, when checked daily. Similarly, reversal of the inhibition of erythroid differentiation has been accomplished. As shown in Table 2, when cells induced with DMSO and treated with IF under the usual conditions were washed and reseeded at high and low density in fresh DMSO- and IF-free medium, amounts of Hb detected in cells that had or had not been treated with IF were comparable. When, however, cells obtained at the fifth day of culture after DMSO+IF treatment, were washed and reseeded into DMSO-supplemented medium, they were fully reinducible by DMSO indicating that IF treatment had not merely selected a DMSO-resistant variant.

Thus our results suggest a marked inhibitory capacity of IF on DMSO-induced Hb production by FLC. It should be emphasised that the decrease of Hb synthesis occurs in conditions where the overall protein synthesis is not affected by IF treatment. This was determined by measuring acid-precipitable radioactivity after pulses or continuous labelling with ³H-arginine (data not shown). The IF-induced inhibition of Hb synthesis is reversed by removal of IF, further

Table 1 Effect of IF on ⁵⁹Fe accumulation, its incorporation into haem and Hb production of FLC cultures

Treatment	⁵⁹ Fe accumulated (c.p.m. per 10^7 cells)	⁵⁹ Fe incorporated into haem (c.p.m. per 10^7 cells)	Hb ($\mu\text{g per } 10^7 \text{ cells}$)
None	13,251	1,421	4.76
DMSO 1.5%	38,402	3,632	47.46
DMSO + IF on day 1	19,922	1,872	5.52
DMSO + IF on day 2	36,435	4,505	29.30
DMSO + IF on day 3	39,649	4,389	25.24

Data shown are for samples obtained at day 5 for culture from a representative experiment. FLC cells were seeded at the density of 10^5 per ml with or without 1.5% DMSO in presence of $0.1 \mu\text{Ci ml}^{-1}$ ⁵⁹Fe-citrate. 1,000 units of IF were added to separate culture samples at time intervals indicated. On day 5, cells were washed, resuspended in 3.0 ml distilled water, and lysed by repeated freeze-thawings. Aliquots of lysates were counted for radioactivity (⁵⁹Fe accumulated). Haem was extracted from lysates according to Scher *et al.*¹⁷ and the radioactivity of such extracts counted (⁵⁹Fe incorporated). Haemoglobin was measured from cell lysates according to Crosby and Furth¹⁸.

Table 2 Reversal of IF inhibition of growth and differentiation of DMSO-stimulated FLC

Treatments	DMSO-treated FLC		DMSO + IF-treated FLC	
	No. of cells per ml ($\times 10^{-5}$)	Haemoglobin ($\mu\text{g per } 10^7 \text{ cells}$)	No. of cells per ml ($\times 10^{-5}$)	Haemoglobin ($\mu\text{g per } 10^7 \text{ cells}$)
5 days of culture	18.3	55.4	5.5	7.2
Cells from above conditions were then treated thus:				
a, Washed and reseeded in DMSO- and IF-free medium at 10^5 cells per ml*	9.2	36.5	9.7	22.7
b, Washed and reseeded in DMSO- and IF-free medium at 10^5 cells per ml*	15.1	37.2	14.8	37.9
c, Washed and reseeded in DMSO-supplemented IF-free medium at 10^5 cells per ml†	15.0	36.4	14.7	42.5

Culture conditions as for Fig. 1. 1,000 Units of IF added on day 1. Haemoglobin measured according to ref. 18.

*Tested 48 h after treatment.

†Tested 96 h after treatment.

confirming the specific character of its effect. The prompt resumption of Hb production in absence of DMSO after IF removal suggests that IF action had not effaced the DMSO imprinting of FLC.

Work is in progress to determine, at the molecular level, the mechanism by which IF addition inhibits Hb synthesis. It is apparent, however, that globin synthesis or its assembly into Hb rather than haem production is the most likely target of IF action.

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Polyclonal Ig production after Epstein-Barr virus infection of human lymphocytes *in vitro*

APART from the specific anti-Epstein-Barr virus (EBV) response in infectious mononucleosis (IM), there is a strong general increase of antibody levels¹. The major increase comprises IgM. As EBV has been shown to infect B lymphocytes selectively² we hypothesised that the virus triggered the infected cells to release immunoglobulin, much like the effect of certain B-cell mitogens³, although the mechanism of activation must be different. It is known that EBV infection *in vitro* of human blood lymphocytes gives rise to increased DNA synthesis⁴ and that lymphoid cell lines carrying the EBV genome shed or release immunoglobulins⁵ of various classes. Furthermore, the IgM increase seen in IM would fit with a polyclonal B-cell activation (PBA) of EBV as PBA usually gives rise to IgM secretion⁶. To test this hypothesis, we have exposed lymphocytes from cord blood and adult peripheral blood to EBV *in vitro* and measured the released IgM, by a double-antibody radioimmunoassay, and individual antibody-forming cells (PFC) against haptenated red blood cells and sheep red blood cells (SRBC).

Lymphocytes, both from EBV seronegative and EBV seropositive donors were infected with strain B95-8 of EBV. Table 1 shows that DNA synthesis, as measured by ³H-thymidine incorporation, increased considerably over the non-infected control cultures, which, however, were stimulated to some degree by the foetal calf serum (FCS) in the medium. The stimulation ratios between infected and control cultures varied from 1.5 to 20 and there was no obvious difference between lymphocytes from cord blood and adults. The amplitude of activation seemed to be specific to each donor, with no correlation with the origin of the lymphocytes. We wished to know whether the B lymphocytes were triggered to become Ig-producing cells, because these infections mimic the *in vivo* situation found in IM. IgM was present from day 3 after infection, as measured by radioimmunoassay, and increased in most cultures by a factor of 10 until day 7 (Table 1). Lymphocytes from adult EBV positive donors secreted detectable IgM in control cultures (Table 1) but at a significantly lower level than in virus-stimulated cultures. The double-antibody radioimmunoassay for IgM was specific for human IgM, and no interference with other human proteins or FCS proteins was observed.

To confirm the radioimmunoassay results, we collected spent media from infected and control cultures and labelled them with ¹²⁵I. Monospecific antibodies against μ , γ , κ and λ chains were used to precipitate out Ig from the bulk of other proteins in the spent medium. The results indicate (Table 2) that the Ig produced is polyclonal, because all

Table 1 IgM production of EBV-infected human lymphocytes after 7 d

Lymphocyte source	(ng IgM per 10 ⁶ cells)*	³ H-thymidine incorporation (c.p.m.)	EBNA induction (% positive cells) †
G.L. adult blood EBV positive	360/30	48520/9056	0.5
G.Li. adult blood EBV positive	290/7	32105/1602	21
N. A-G. adult blood EBV positive	30/0	ND/1225	1.5
K.H. adult blood EBV negative	460/ND	ND/1510	8
I.E. adult blood EBV negative	115/30	1910/ND	ND
J.E. adult blood EBV positive	245/ND	6147/946	4
B.A. adult blood EBV positive	250/125	ND	ND
A.P. adult blood EBV positive	54/8	8112/1952	5
K.G. adult blood EBV positive	160/84	128442/8267	17
N.C. IM-blood EBV positive	9/0	9612/812	0.5
P.H. cord blood EBV negative	10/0	25878/5983	9
A.Z. cord blood EBV negative	960/0	68741/7357	12
E.K. cord blood EBV negative	ND	140878/19827	14

Figures in columns 2 and 3 are infected over control.

*Sensitivity of radioimmunoassay: 3 ng ml⁻¹. ND, not done.

†Values from infected cultures; control cultures were always negative.

Lymphocytes were separated on Ficoll-Isopaque¹⁷. Two batches of EBV (supernatant of B 95-8 cells (ref. 7) filtered through a Millipore membrane pore size 0.45 µm) were used. Lymphocytes were pelleted and resuspended in B 95-8 supernatant (1 ml was added to 10⁶ cells). The cells were incubated for 1 h at 37 °C with continuous shaking. Thereafter the cells were washed three times with medium (RPMI 1640, supplemented with 10% FCS and antibiotics), finally they were resuspended in medium to a concentration of 10⁶ cells per ml. Cells were incubated for 7 d at 37 °C in 5% CO₂ and 95% air in 50-ml plastic bottles (Falcon plastics). 16 h before collection 10⁶ cells were transferred into Falcon microplate wells (2 × 10⁵ cells in each well) and 1 µCi ³H-thymidine (Amersham, specific activity: 5 Ci mmol⁻¹) was added to each well. The cultures were sucked off with the aid of multiple sample processor (Skatron, Norway) to glass fibre filters. After drying, the filters were placed in vials containing scintillation fluid. The isotope determinations were made by a Packard Tri-Carb scintillation spectrometer and expressed as c.p.m. EBV-determined nuclear antigen (EBNA) staining was carried out on day 7 according to the method of Reedman and Klein¹⁸. A double-antibody radioimmunoassay was established for measurements of minute amounts of IgM. IgM was isolated from a pool of human serum by precipitation with 50% saturated ammonium sulphate, washed three times with 33% saturated ammonium sulphate, dialysed against 0.1 M Tris-HCl buffer, pH 7.4, containing 1 M NaCl and fractionated on Sephadex G-200 in the same buffer. The first peak was pooled and fractionated by Pevicon block electrophoresis in Veronal-HCl buffer, pH 8.6, $I=0.1$. Isolated IgM was kept in a 0.1% glycine buffer in phosphate buffered saline, pH 7.4, to avoid aggregation. The purified IgM was labelled with ¹²⁵I according to a modification to Hunter and Greenwood¹⁹. Radioimmunoassay was performed as follows: 10 µl spent medium or standard (purified IgM) in twofold dilutions from 25 to 0.05 ng was incubated for 4 h, 37 °C with 10 µl of rabbit anti-IgM (Dakopatts, Copenhagen), diluted 1:4,000 plus 25 µl of normal rabbit serum diluted 1:500. Then 10 µl of labelled purified IgM (0.5 ng labelled with 5,000–10,000 c.p.m.) added and the mixture incubated for 1.5 h at 37 °C. 25 µl of sheep anti-rabbit globulin 1:50, was then added for 18 h of incubation at 4 °C. All reagents were diluted in a 10 mM Tris-HCl buffer, pH 7.5, containing 1 mM EDTA, 15 mM NaCl and 0.4% Triton X-100 and 20 mg bovine serum albumin (BSA) per ml. Precipitates were washed three times in cold buffer without detergent and BSA and counted in an Intertechnique γ-counter. A standard curve was plotted and the concentration of IgM of the unknown samples from the spent medium of infected and non-infected cultures were determined according to the standard curve. A similar radioimmunoassay for Ig light chains has recently been described by Rosén *et al*²⁰.

four antisera showed significant precipitation values. Autoradiographic analysis of the radioactive precipitates on slab gels (4–25% gradient sodium dodecyl sulphate-polyacrylamide gel, in non-reducing conditions) confirmed two facts: 19S IgM was synthesised and both IgG and IgM were produced by the stimulated cells (unpublished results of A. R. and R. Laskov). The control culture precipitates showed weak bands in the positions of IgM and IgG but the quantitative differences were striking.

We have looked for several antibody specificities, including the heterophile antibody in the immunoglobulins from the supernatants of the EBV-stimulated cultures, but without success. However, when testing for single antibody forming cells (Table 3) in a modified plaque assay* after 6 d of culture, we found that EBV-exposed cord blood lymphocytes generated direct PFC against both haptenated RBC and SRBC. The frequencies were of the same order as with cells exposed to 20 µg of pokeweed mitogen. Single antibody-forming cells were developed following the principles described by Jerne and Nordin²¹, but essentially with the modifications for human effector cells described by Fauci and Pratt²². Purified blood lymphocytes (5 × 10⁶) were spun down and incubated in 1 ml of supernatant of B 95-8 for 60 min at 37 °C. After two washes the cells were incubated

in flasks (Falcon 3013) at 10⁶ per ml in RPMI in 10% FCS and 5% CO₂ in air. After 3–7 d the cells were collected and plaqued on thin-layer agarose as described by Bullock and Möller¹⁰. The target cells were SRBC or haptenated (FITC) SRBC (ref. 11). Only direct PFC were studied. Guinea pig serum diluted 1:40 was the source of complement. As a B-cell mitogen, 20 µg of pokeweed mitogen was added to 5 × 10⁶ lymphocytes for the whole culture period.

Between 50 and 90% inhibition of the number of plaques formed was observed when a rabbit anti-IgM antiserum was added before plaques were developed. This indicated that IgM antibodies caused plaque formation.

EBV nuclear antigen (EBNA) (ref. 18) was induced as a result of infection (Table 1). The appearance of EBNA agrees with the assumption that the stimulation of B cells was due to the penetration of EBV into the cells.

We have demonstrated that peripheral blood lymphocytes from cord blood and adults make immunoglobulins after exposure to EBV *in vitro*. The impact of this finding is threefold. First, we believe that it represents an *in vitro* correlate of the *in vivo* infection with EBV in IM¹² because there is an increase of polyclonal IgM in this disease¹. Our demonstration of IgM and IgG with κ and λ chains by immunoprecipitation and single PFC with at least two antibody specificities (anti-FITC and anti-heterologous RBC) suggests polyclonality of these antibodies induced *in vitro*. Ig secretion has been reported², by established lymphoid lines made immortal by the addition of EBV¹³. This study, however, reveals the early events, before the establishment of a line.

Our data show that human newborn and adult peripheral blood contains cells that can be induced to produce antibodies. Antibody-producing potential is thus not confined to stationary cells of lymphoid organs, and age does not seem to influence this capacity at least with regard to IgM

Table 2 Precipitation of Ig from ¹²⁵I-labelled supernatants

Antibody	Donor 1	% Precipitated*	Donor 2
Anti-μ chain	1.0		1.0
Anti-γ chain	0.8		1.2
Anti-κ chain	0.3		0.3
Anti-λ chain	0.4		0.3

The precipitation systems were optimised by titrating purified IgM or IgG against 50 µl of the rabbit antisera.

*Infected minus control.

Table 3 Number of antibody-forming cells (PFC per 10^6 recovered cells) in human blood lymphocytes after *in vitro* exposure to EBV and pokeweed mitogen

Material added <i>in vitro</i>	Cell source	EBV*		Pokeweed mitogen†		None	
		FITC-SRBC-PFC	SRBC-PFC	FITC-SRBC-PFC	SRBC-PFC	FITC-SRBC-PFC	SRBC-PFC
Cord blood	1	140	40	180	110	0	0
	2	210	65	235	160	15	0
	3	95	30	125	70	0	0
	4	180	45	140	100	10	0
Adult blood	1	165	40	280	165	25	0
	2	85	35	175	170	0	0
	3	240	155	270	235	15	15
	4	260	230	295	180	0	0

Plaque assay was done after 6 d culture, both against FITC-coupled sheep red blood cells (FITC-SRBC-PFC) and SRBC alone (SRBC-PFC).
 *A 1-ml sample of EBV from B 95-8 supernatant was added to 5×10^6 in 2 ml of purified blood cells from umbilical cord and adult donors. of unknown immune status to EBV. The mixture was incubated for 1 h at 37 °C and the cells were washed and cultured.

†20 µg pokeweed mitogen was added at start of culture.

antibodies. Finally, we believe that EBV activation will be helpful in the study of B-cell triggering as the virus binds to B lymphocytes by means of receptors that are intimately associated with the complement receptors¹⁴. There is still controversy^{15,16} as to whether mere binding to the complement receptor will result in full activation of the B lymphocytes. We intend to explore this area, utilising the same experimental system, by exposing blood B lymphocytes to both non-transforming and heat inactivated EBV, respectively, both of which supposedly bind to B lymphocytes by means of the same receptor as normal EBV. We also plan to investigate whether the antibody-forming cell is EBNA positive in these conditions and, if so, whether an EBNA positive B cell can make anti-EBV antibodies.

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Prevention of oncornavirus-induced sarcomas in cats by treatment with antiviral antibodies

In mice, infections with leukaemogenic viruses, as well as subsequent development of disease, can be permanently suppressed by treatment with antibodies specific for the major glycoprotein (gp71) of Friend murine leukaemia virus (MuLV Friend)^{1,2}. Similar protocols using antibodies

to feline leukaemia virus (FeLV) result in elimination of FeLV infection in 6-month-old kittens. Furthermore, FeLV-infected kittens became non-viremic after treatment with antibodies to the murine virion glycoprotein³. This finding indicates that the interspecies portions of C-type virus glycoproteins can be suitable targets for immunotherapeutic regimens. It was of interest to extend the studies with leukaemia viruses to agents which induce other classes of neoplasms. We describe here a study of the effect of antibody therapy on infections in cats with feline sarcoma virus (FeSV). Our preliminary results indicate that antibody therapy can be effectively applied for prevention of virus-induced solid tumours. Moreover, this tumour model may be more attractive and amenable to study than virus-induced leukaemia.

The virus used in these studies was the Snyder-Theilen strain of FeSV. Kittens were injected with aliquots from a single pool of cell-free tumour extracts containing between $10^{3.2}$ and $10^{4.2}$ focus inducing units (FIU) representing about 10^3 and 10^4 cat LD₅₀ units respectively.

The antisera used have been described in detail elsewhere^{2,3}. Briefly, goat antiserum to FeLV ('FeLV serum') neutralised the homologous virus to a high titre (1/8,000) and was highly cytotoxic in a Cr-release test for FeLV producing F422 cells (titre 1/8,000). This serum was absorbed with foetal calf serum and normal cat antigens before use. Goat antiserum to MuLV Friend gp71 ('MuLV gp71 serum'), also prepared in goats, neutralised FeLV to a titre of 1/400 and was cytotoxic for F422 cells to a titre of 1/500. Total globulins of both sera were obtained by precipitation with ammonium sulphate. The IgG fraction of MULV gp71 serum was then isolated by chromatography on DE-52. The respective globulin fractions and MuLV gp71 IgG were used at a concentration of $5 \times$ with respect to whole serum volume.

Three-week-old kittens from a specific pathogen-free colony were infected subcutaneously (s.c.) in the scapular region with the cell-free FeSV tumour extract. Six or 12 d later, whole serum, globulins or IgG were administered once intraperitoneally followed by seven s.c. injections over a period of 16 d (see Table 1). In Experiment I, all kittens were from the same mother, whereas the other studies were with randomised animals.

As can be seen from the effect on the 11 control animals, represented by untreated animals or groups which received normal goat serum globulins, the FeSV tumour extract was lethally oncogenic in our system. All animals died after development of progressive sarcomas with many secondary growths which probably, in large measure, represent new primaries induced by FeSV. The mean survival period for kittens infected with $10^{3.2}$ FIU was 53 ± 9.4 d, whereas the animals receiving the higher virus dose died somewhat earlier.

Of the two antibody treatments employed, the outcome with FeLV-serum globulins was more effective. When the

Table 1

Exp. No.	Virus dose (FIU)	No. of cats	Treated with	Amount (ml) injected; days p.i.	Tumour detected at days p.i.	Tumour growing to size of	Complete regression at weeks p.i.	Survival period of cat (days)	Number and % of animals recovered
I	$10^{4.2}$	2	FeLV serum globulin	11 ml 6-22	(a) 15 (b) 15	Hazelnut Hazelnut	4 4	> 285 90†	2 (100)
		2	Normal goat globulin	11 ml 6-22	(a) 23 (b) 23	P with S*	NA†	30 28	None
II	$10^{3.2}$	5	FeLV serum globulin	11 ml 6-22	No Tumour	No Tumour	NA	all > 211	5 (100)
		4	Normal goat globulin	9 ml 6-22	(a) 20 (b) 38 (c) 36 (d) 30	P with S " " "	NA " " "	47 63 55 48	None
		3	—	—	(a) 34 (b) 20 (c) 30	P with S " "	NA " "	60 51 37	None
		1	FeLV serum globulin	11 ml 12-28	No Tumour	No Tumour	NA	> 110	1 (100)
		5	MuLV gp71 Serum IgG	11 ml 6-22	(a) No Tumour (b) 30 (c) 19 (d) 28 (e) 30	No Tumour Hazelnut P with S " "	NA 6 NA " "	> 110 49 41 41	2 (40)
IV	$10^{3.2}$	2	Normal goat serum IgG	11 ml 6-22	(a) 48 (b) 50	P with S "	NA "	50 68	None

* P with S—progressive with secondary growths.

† NA—not applicable.

‡ This animal died of enteritis; no tumour evident.

treatment was initiated 6 d post-infection (p.i.), none of the animals died as a consequence of tumour development. The single animal in Experiment I which died 90 d p.i. was lost due to enteritis, and showed no evidence of tumour. In Experiment III, where only one kitten was tested, no tumour developed even when the antibody treatment was initiated as late as 12 d p.i.

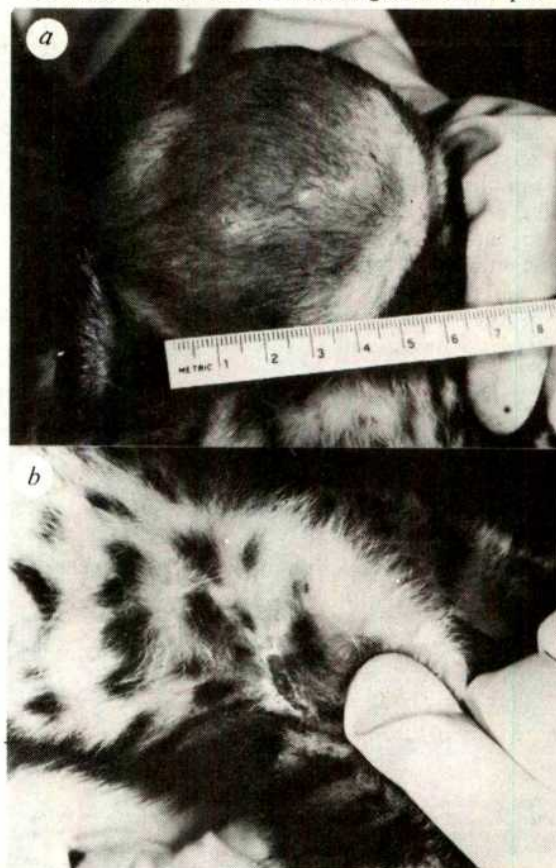
Kittens in Experiment I, receiving the higher virus dose, developed clearly palpable tumours at the site of FeSV inoculation during antibody treatment. In contrast to the situation in the control animals these tumours completely regressed. After antibody treatment, scars remained in the skin at the site of virus inoculation which were surrounded by pigment-free hairs (Fig. 1b). This occurred regardless of whether palpable tumours were detected. Thus far, no indication has been obtained that any of the surviving animals have developed new tumours (in Experiment I this represents nearly 285 d p.i.).

Some protection against FeSV tumour development was also achieved with the MuLV gp71-IgG, although in this study (Experiment IV), only two of five kittens survived. One of the surviving animals developed a visible tumour which subsequently regressed. Thus, interspecies MULV gp71 antibody can be effective against solid tumours induced by FeSV. Additional studies using larger amounts of IgG and other protocols are in progress to improve the effect of the treatment.

Our previous studies in kittens cured of FeLV infection by antibody treatment revealed that the non-viremic animals resisted subsequent challenges with FeLV and developed significant neutralising titres against the virus³. Some of these animals received three further challenges with live FeLV and one with FeSV. One female cat from this group of hyperimmunised animals was mated and subsequently delivered three kittens which were challenged with $2 \times 10^{3.2}$ FIU of FeSV tumour extract at 22 d of age. These animals had not developed any palpable tumours at 70 d p.i.

The prime features of these preliminary studies can be

Fig. 1 a, Tumour of a control cat infected with $10^{3.2}$ FIU of feline sarcoma virus: 60 d p.i. b, Scar of a cat infected with $10^{3.2}$ FIU and treated with FeLV serum globulin: 43 d p.i.



summarised as follows: (1) Protection against death from development of progressive FeSV-induced tumours can be achieved by administration of globulin fractions from goats immunised with (a) FeLV and (b) MuLV gp71. (2) In some cases, animals protected by the antibody treatment developed sizeable tumours at the site of virus inoculation. But, these tumours regressed while control cats died of progressive sarcomas. (3) Scar formation at the site of FeSV inoculation was observed in several cases in recovered animals. (4) Preliminary results indicate that transfer of immunity from mother to offspring may be possible.

The system described above seems to represent a classical model for study of tumour growth and regression in the cat in response to external immunological controls. It is certainly remarkable that by simple application of antibody, it is possible to achieve such a striking effect on a solid tumour without any obvious detriment to the host. Of particular interest are the cases where the tumour was detectable by palpation during the course of antibody treatment, suggesting that a direct interaction of the heterologous antibody with the growing tumour may occur. The actual mechanism by which the tumour is eliminated and the significance of the residual scars are under investigation. Our previous experience in the mouse and cat indicates that an immune response by the host was required for long-term protection against virus infection and/or leukaemia development. In the present system, such an immune response could be directed against prominent cell surface antigens including virus structural components and/or FOCMA (ref. 4). Further studies are required to determine if such host immunity is in fact activated, and whether it is sufficient to guarantee long-term protection against sarcoma. The transfer of immunity from mother to offspring reported also by others in the cat^{5,6} as well as in the mouse^{7,8} is an important phenomenon. The nature and specificity of the antibodies primarily involved, however, are not yet known.

The results presented above together with other studies in the mouse and cat are highly promising and suggest possible application of antibody therapy to other virus-induced malignancies. Of special interest in this regard is the interspecies activity of the antibody *in vivo*. This raises the possibility that tumours in widely differing species expressing cross-reactive glycoprotein antigens will be amenable to similar studies, using well-characterised reagents such as anti-MuLV gp71 antisera.

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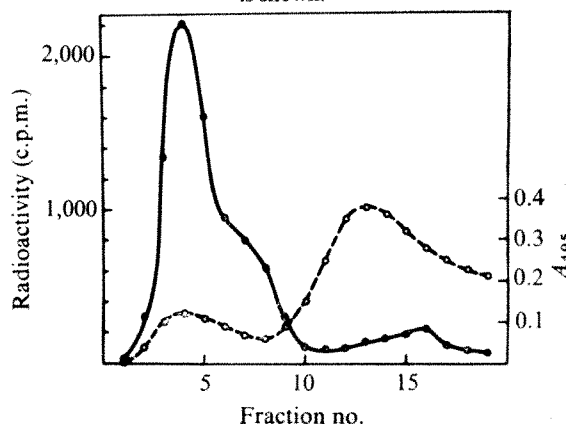
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Melanotropin-daunomycin conjugate shows receptor-mediated cytotoxicity in cultured murine melanoma cells

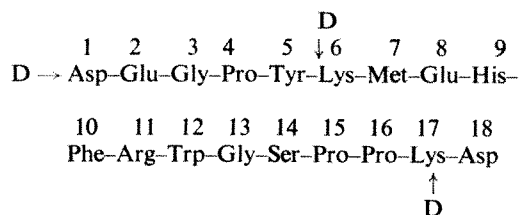
MELANOTROPIN (MSH) binds to specific receptors at the surface of mouse melanoma cells, preferentially in the G-2 phase of the cell cycle¹. On prolonged incubation, a fluorescein-MSH conjugate is internalised and appears intracellularly in small vesicles². The same phenomenon has been demonstrated by electron microscopy with an MSH-ferritin-fluorescein conjugate³. Although a role for the internalisation of MSH receptors in the execution of the hormonal message is still open to question, the fact that these conjugates of MSH are specifically recognised and internalised by melanoma cells offers a possibility for specific, site-directed chemotherapy of melanomas. We have therefore assumed that MSH-toxin conjugates might be specifically recognised and internalised and thereby have a selective toxic effect on cells that display MSH receptors on their surface. Even though the principle of site-directed chemotherapy has been known for a long time, it is only recently that this field became active⁴. Conjugates of specific antibodies and cytotoxic substances⁵⁻⁷, as well as a DNA-daunomycin complex⁸ have been used in an attempt to increase the selectivity of the toxic agents. In this report we describe the synthesis of a melanotropin-daunomycin conjugate and its MSH receptor mediated cytotoxic effect on mouse melanoma cells *in vitro*.

A melanotropin-daunomycin conjugate was prepared using the procedure developed by Hurwitz *et al.*⁹ for the covalent binding of daunomycin to antibodies. Daunomycin 20 mg (380 μ mol) (Sigma) was dissolved in 1.5 ml phosphate-buffered saline, pH 7.4 (PBS). NaIO₄ 11.5 mg (540 μ mol) was added and the mixture was incubated at 22 °C in the dark for 1 h. The excess NaIO₄ was neutralised with glycerol, added to a 50 mM final concentration. 30 mg β -MSH (prepared by us¹⁰) containing 1 μ Ci of ¹²⁵I-MSH (1) as a marker was added in 1 ml of 0.15 M K₂CO₃ (pH 9.5). The mixture was incubated for 1 h at 22 °C. The resultant conjugate was reduced by NaBH₄ (0.3 mg ml⁻¹) at 37 °C for 2 h. The reaction mixture was applied to a Sephadex G-25 (90 cm $\times$ 2 cm) column and eluted with PBS. Radioactivity of the fractions was measured by a γ -counter and the concentration of daunomycin was monitored spectrophotomerically at 495 nm. The appearance of the red colour in the void volume, coincident with the major peak of ¹²⁵I-MSH indicated the presence of a melanotropin-daunomycin conjugate (Fig. 1). On the basis of

Fig. 1 Separation of melanotropin-daunomycin on Sephadex G-25. A reaction mixture of melanotropin-daunomycin was applied to a Sephadex G-25 column as described in the text. Radioactivity (●) and absorbancy at 495 nm (○) of the fractions is shown.



the specific radioactivity and absorbancy at 495 nm of the major fraction, the molar ratio of daunomycin to MSH in the conjugate was calculated to be 2.2:1. The purity of the compound was tested by high voltage electrophoresis. The purified conjugate was hydrolysed by 6 N HCl at 110 °C for 18 h and the amino acid composition was determined, parallel with the hydrolysate of a mixture of daunomycin and unconjugated β -MSH. About 50% of lysine and aspartic acid were recovered from the conjugate indicating that the oxidised product of daunomycin (D) had substituted the ϵ -aminolysyl residue(s) (no. 6 and/or no. 17) and the terminal amino group of aspartic acid. This suggests the following general formula for the melanotropin–daunomycin conjugate:



Efforts to delineate substitution of lysyl residues by analysis of tryptic and chymotryptic hydrolysates were unsuccessful.

The cytotoxic index of daunomycin was 0.99 when the antibiotic was added in 4 μM concentration to cultures of melanoma cells. Melanotropin-daunomycin showed the same level of toxicity at 1.4 mM (Table 1). On a molar basis, therefore, the daunomycin-MSH conjugate is about three times more toxic to melanoma cells than free daunomycin. MSH, daunomycin and melanotropin-daunomycin conjugate markedly changed the morphology of melanoma cells. With daunomycin, cells develop large nuclei, most probably due to a G-2 block, similar to that observed in other systems¹¹; surviving cells showed no dendritic processes. In the presence of melanotropin-daunomycin, mostly cells with large nuclei were found with normal or fragmented processes. The median cell volume in the control cultures was 1,450 μm^3 ; in the presence of MSH 1,500–2,000 μm^3 ; and with melanotropin-daunomycin, 1,700–2,600 μm^3 . Although free daunomycin was as toxic to 3T3 cells as to melanoma cells, melanotropin-daunomycin (1.4 μM) was not toxic to 3T3 fibroblasts (Table 1). Neither MSH (0.2 mM)

nor MSH–daunomycin (1.4 μ M) had significant effect on the morphology or size of 3T3 cells. The toxic effect of melanotropin–daunomycin can be prevented by free MSH; free MSH has no effect on the toxicity of daunomycin (Table 1).

Daunomycin or melanotropin–daunomycin conjugate was added to coverslip cultures of melanoma cells and of 3T3 cells and the uptake of daunomycin was monitored by fluorescence microscopy. After 1 h of incubation at 37°C, internalisation of daunomycin was detected by fluorescence microscopy with both melanoma and 3T3 cells (Fig. 2*a,b*). The daunomycin–melanotropin complex was internalised by melanoma cells only (Fig. 2*c,d*) and this process could be blocked by free melanotropin (Fig. 2*e*). Melanotropin had no effect on the uptake of daunomycin by melanoma cells (Fig. 2*f*) or by 3T3 cells (not shown).

Thus we have synthesised a melanotropin–daunomycin conjugate which is more toxic to mouse melanoma cells than daunomycin alone and in the same concentration range the conjugate is not toxic to mouse 3T3 fibroblasts. This increase in selective toxicity is remarkable because unconjugated daunomycin is equally toxic to both cell lines (Table 1); the same cytotoxicity of free daunomycin was found in other systems^{8,9}. Because both the toxic effect and uptake of melanotropin–daunomycin in melanoma cells can be partially prevented by free melanotropin (Table 1 and Fig. 2), we assume that recognition of melanotropin–daunomycin by MSH receptors, present on melanoma cells¹ but absent from 3T3 cells (unpublished work), is responsible for the enhanced selective toxicity.

As we could demonstrate by fluorescence microscopy, after 1 h of incubation, the fluorescence of unconjugated daunomycin (or the fluorescence of the aglycone) appears in the nuclei of both melanoma and 3T3 cells.

In contrast, melanotropin–daunomycin appears in melanoma cells only. Whether the conjugate reaches the nucleus intact or whether it is degraded, is not known. The mechanism of penetration of melanotropin–daunomycin into melanoma cells is probably similar to the endocytosis we observed with fluorescein–MSH (ref. 2) and with fluorescein–ferritin–MSH (ref. 3). These conjugates as well as ^{125}I –MSH (ref. 12) bind in patches on the surface of melanoma cells at 0 °C (refs. 2, 3, 12). At 37 °C, FITC–MSH appear in vesicles² and the MSH–ferritin–FITC conjugate in premelanosomes in a few hours. We assume that endocytosis of these conjugates resembles phagocytosis by

Table 1 Toxicity of melanotropin-daunomycin on melanoma and 3T3 cells

Cells	Additions to culture medium	Cytotoxic index*
Melanoma	None	0.00
	Daunomycin, 4 μ M†	0.99
	Daunomycin, 4 μ M + melanotropin, 200 μ M	1.00
	Melanotropin, 2 μ M	0.05
	Melanotropin–daunomycin, 0.35 μ M	0.65
	Melanotropin–daunomycin, 0.70 μ M	0.87
	Melanotropin–daunomycin, 1.40 μ M	0.98
	Melanotropin–daunomycin, 1.40 μ M + melanotropin, 4 μ M	0.57
	Melanotropin–daunomycin, 1.40 μ M + melanotropin, 40 μ M	0.47
3T3	Melanotropin–daunomycin, 1.40 μ M + melanotropin, 200 μ M	0.28
	None	0.00
	Daunomycin, 4 μ M†	0.99
	Melanotropin, 2 μ M	0.01
	Melanotropin–daunomycin, 1.4 μ M	0.02

Cloudman S-91 melanoma cells (NCTC 3960) were inoculated in 25 ml Falcon flasks and cultivated at 37 °C in an atmosphere containing 5% CO₂, in Ham's F-10 medium supplemented with 5% foetal calf serum and 2 U ml⁻¹ gentamycin. The mouse 3T3 fibroblast cell line (from Dr Paul Lebowitz) was cultivated in Earle's MEM containing 5% foetal calf serum, 100 U ml⁻¹ penicillin and 100 U ml⁻¹ streptomycin. After 1 d of growth, the medium was changed to a fresh one containing additions as listed. After 3 d in culture, the cells were rinsed with fresh medium and removed from the flasks by shaking, after treatment with EDTA in Tyrodes solution¹. The cells were pelleted by centrifugation (1,000*g*, 5 min.) and washed with serum-free medium. The number and size of the cells was determined with a Coulter 'channelyser'. Cell viability was determined by a modification of the dye exclusion test¹⁴. Four parallel flasks were used for each point.

$$\text{*Cytotoxic index} = \frac{(\text{no. viable cells in control culture} - \text{no. viable cells in treated culture})}{(\text{no. viable cells in control culture})}$$

†Minimum dose of daunomycin required for maximum cytotoxicity. This was determined from dose-response curves and the values for melanoma and 3T3 cells were similar to those observed with other cell types^{8,9}.

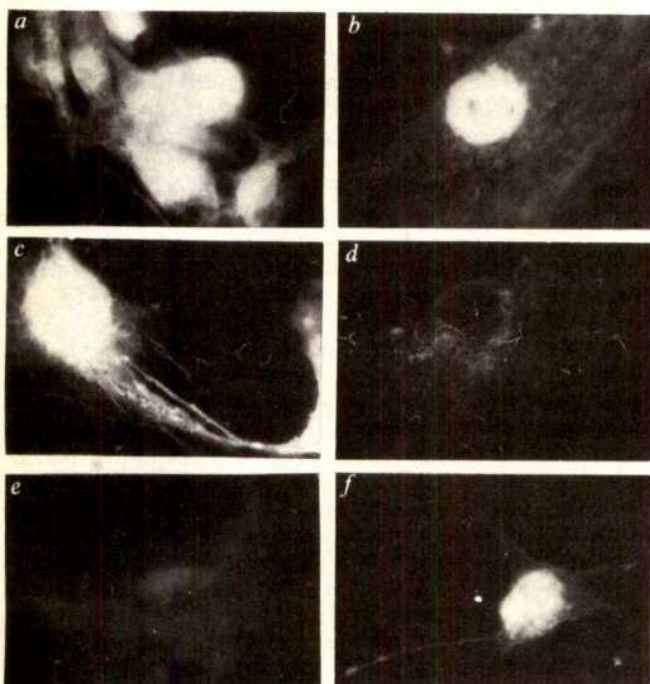


Fig. 2 Fluorescence microscopy of daunomycin and melanotropin-daunomycin treated cells. Melanoma cells and 3T3 fibroblasts were grown for 2 d in Ham's F-10 medium on coverslips. Before treatment, the cultures were rinsed with fresh medium; then incubated at 37 °C for 60 min as follows: *a*, melanoma cells were incubated with 4 μM daunomycin; *b*, 3T3 cells with 4 μM daunomycin; *c*, melanoma cells with 1.4 μM melanotropin-daunomycin; *d*, 3T3 cells with 1.4 μM melanotropin-daunomycin; *e*, melanoma cells with 1.4 μM melanotropin-daunomycin + 0.1 mM melanotropin; *f*, melanoma cells with 4 μM daunomycin + 0.1 mM melanotropin. After incubation, cells were rinsed with serum-free medium (0 °C), fixed with 2% paraformaldehyde for 5 min (0 °C), rinsed with distilled water and air-dried. Preparations were mounted with Leitz immersion oil and cellular localisation of the red fluorescence (white areas on the microphotographs) due to the presence of free daunomycin or melanotropin-daunomycin was detected by fluorescence microscopy by using a Leitz Diavert microscope equipped with a 100 W mercury burner and a Ploem epi-illuminator.

macrophages¹³ suggesting that MSH receptors may play a similar role on melanoma cells as Fc receptors do on macrophages: the formation of a ligand receptor complex may signal endocytosis. Accordingly, the absence of MSH receptors and permeability barrier to MSH in 3T3 cells would explain lack of endocytosis of the hormone-toxin conjugate which may be responsible for the resistance of 3T3 cells to melanotropin-daunomycin.

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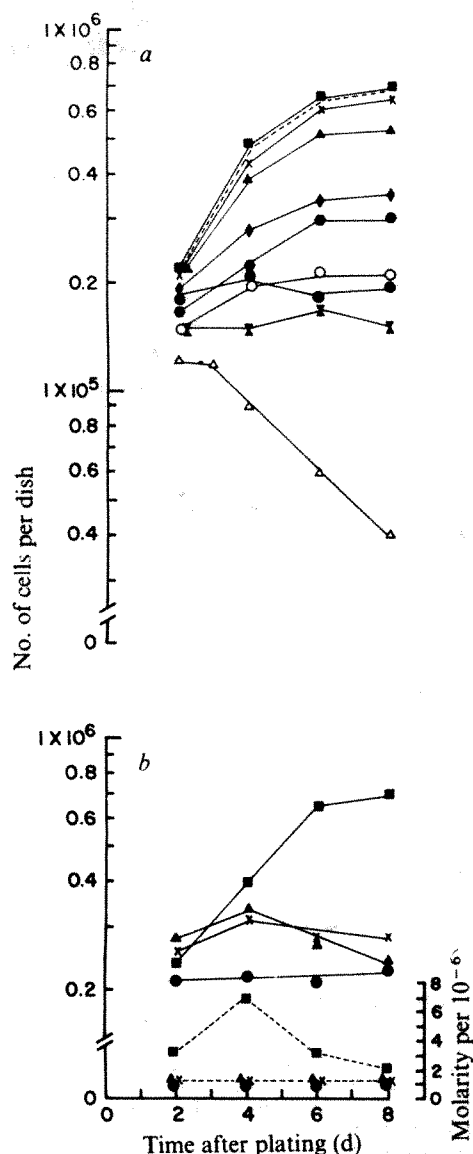
Procarcinogen activation and hormonal control of cell proliferation in differentiated primary adult rat liver cell cultures

CONCERTED interactions among hormones and nutrients control animal cell proliferation¹. For example, in the rat, insulin, glucagon, glucocorticoid, parathyroid hormone, iodothyronines and amino acids regulate normal hepatic proliferation—a prominent system for studying epithelial cell growth control². Endocrine and nutritional factors also modify the efficiency with which chemicals produce experimental tumours in these animals notably, the induction of hepatomas by aromatic amines³. We report here observations with a novel primary monolayer adult rat liver culture system which may prove helpful towards studying these interactions under defined conditions⁴. These cells proliferate in response to hormones and nutrients; they maintain differentiated hepatic functions such as albumin and arginine biosynthesis for at least 8-10 days after plating; and they metabolise the procarcinogen *N*-2-acetylaminofluorene (AAF) into a proximate form, *N*-hydroxy-AAF (ref. 5).

Cell isolation procedures and plating methods (which use arginine-free medium to select for arginine-synthesising hepatocytes⁶) are given in Fig. 1 (legend). About 10% of the cells in 5-7 g of normal liver are recovered for plating. Over 95% of these cells are viable (measured by trypan-blue exclusion) and hepatocyte-like with respect to size ($\geq 25 \mu\text{m}$ diameter, measured with an electronic particle counter), to histochemical staining properties (positive for glycogen and glucose-6 phosphatase), and to ultrastructure visualised by electron microscopy (glycogen granules, microbodies, rough endoplasmic reticulum; K. Dempo *et al.*, in preparation). Similar characteristics are retained 24-48 h post-plating by the surviving cell fraction, attached to the plastic substratum, which represents about 10-20% of the initial inoculum and which begins to form monolayer aggregates⁶ at this time (see ref. 4 for photomicrographs). The recovery efficiency⁶ of 10-20% is invariant with inoculum concentrations of 10^5 - 10^6 cells per dish. Cell survival after 72-96 h requires serum (Fig. 1a) without which cells randomly detach (floating cells are dead and eventually most lyse).

Growth curves are shown in Fig. 1. Serum alone fails to sustain significant proliferation rates but if hormones and inosine⁷ are added, especially hydrocortisone or insulin, proliferation is stimulated (Fig. 1a). Titration studies suggest synergistic hormone interactions; stimulation is detected at concentrations as low as 50 ng ml^{-1} . Analogues with negligible potency (for example desAla, desAsp-insulin⁸ or androstenedione⁹) are non-stimulatory. Net increased proliferation, which absolutely requires exogenous ornithine, is otherwise satisfied by 0.4 mM arginine but not by either exogenous putrescine (0.008-0.4 mM) or 8 μM arginine (Fig. 1b). DNA synthesis rate and mitotic kinetic patterns are similar to those reported for foetal hepatocytes¹⁰. Under optimal conditions (Fig. 1a), adult log-phase cultures (96-120 h post-plating) contain at least $46 \pm 2\%$ (s.e.m.; $N = 3$) of cells in S-phase, measured radioautographically¹⁰ following a 16-h pulse with ³H-dT or as estimated by flow microfluorimetric methods⁷.

Similar growth curves are obtained if 24 h cultures receive fresh 24 h cell-free 'conditioned medium'; therefore, enhanced attachment or cell-settling do not cause increased cell numbers.



Serum contains additional stimulatory material for if slowly growing cultures are established in 'complete medium' with 1% serum, then increasing amounts of serum added 24–48 h post-plating produce a dose-dependent family of increasing growth curves (not shown). Triiodothyronine or glucagon (Fig. 1a), and FGF (ref. 11), or EGF (ref. 12) (0.01–1.0 μg ml⁻¹) do not stimulate growth and thus do not seem to be limiting under these conditions (see discussion in ref. 13). Although cell proliferation in this adult system is more dependent than a similar foetal system upon exogenous ornithine¹⁴ and hydrocortisone¹, it is not yet clear if this results from different intrinsic cellular properties and/or from different initial physiological states.

³H-ornithine is a useful probe of specific and general metabolic functions in liver cell cultures⁶ (Fig. 2). These studies indicate that cultured adult cells synthesise albumin and arginine because first, ³H-ornithine enters albumin and cellular macromolecules which, after acid hydrolysis, contain >80% of the protein-associated radioactivity in the form of ³H-arginine (not shown), and second, incorporation is inhibited ≥98% by cycloheximide (5 μg ml⁻¹; not shown). The immunoprecipitation procedure used¹⁵ to measure albumin biosynthesis accurately reflects relative rate differences of albumin production because an independent previously validated radioimmunoassay¹⁵ gives similar results to those shown in Fig. 2. Thus, day 2 and 8 rates measured by radioimmunoassay are 110 and

Fig. 1 Hormonal control of cell proliferation in adult liver cell cultures. Fisher/344 male or female rats (150–200 g) were ether anaesthetised at 0900–1200 h. An abdominal incision was made extending into the thoracic cavity; the rat was exsanguinated by cardiac puncture and its intestinal contents resected. A 20 ml plastic syringe equipped with a 25 gauge × 1 inch needle was inserted into the portal vein, clamped distally to splanchnic and gastric veins, and the superior vena cava was cut. The liver was perfused 2–3 min with 20 ml 'digestion buffer.' This solution contains (in g l⁻¹) Type I Sigma collagenase, 3.0; NaCl, 7.597; KCl, 0.224; NaH₂PO₄·H₂O, 0.138; D-glucose, 1.802; phenol red, 0.0012; insulin (from W. Bromer, Eli Lilly), 0.01; and hydrocortisone-succinate and inosine (Sigma), 0.01 each; it is prepared fresh, adjusted to pH 7.4, sterilised with a 0.45 μm Nalgene filter, and preincubated 1–4 h at 37 °C. Two perfused excised livers were freed of extraneous tissue and washed twice with 10 ml 'complete medium' (arginine-free Dulbecco-Vogt's modified Eagle's medium, supplemented with 0.4 mM L-ornithine, 15% v/v heat inactivated dialysed foetal bovine serum⁶ and 10 μg ml⁻¹ each of glucagon, T₃ and of hormones and purine present in digestion buffer). 'Basal medium' is complete medium minus hormone and purine supplements. Tissue was dissected into 5 × 8 mm pieces, washed twice, pooled and incubated at 37 °C with 40 ml aliquots of digestion buffer in a 125 ml Erlenmeyer flask (four 10-min treatments; 5 cm × 7 mm magnetic stirring bar and Magne-Stir Model No. 10 stirrer, rheostat control 35–40). Cell suspensions after each treatment were transferred to a collecting flask containing 10 ml 'complete medium' at 0–4 °C. Ice-cold medium (160 ml) was mixed into the final pooled cell suspension; 40 ml aliquots were dispersed into eight 50 ml tubes (Falcon) and centrifuged at 150–200g for 13 s. Pellets were gently resuspended in serum-free 'complete medium' and recentrifuged. Resulting pellets were vigorously resuspended and titrated with a Coulter counter (threshold > 10 μm; attenuation, 1; aperture, 256). A third centrifugation step was repeated and the final pellets resuspended into appropriate plating media. Cells were plated (10⁶ per 2 ml medium per 35 mm Falcon plastic tissue culture dish) and cultured, without media changes, in an humidified 10% CO₂-air incubator at 37 °C. Cell counts (numbers of attached cells removed by trypsin) and amino acid analyses were performed by previously validated methods^{6,16}. Counting errors were ±2–10% (N = 3). a, Cell growth curves under various plating conditions: complete medium, male (■) or female cells (□) or female cells (■) or female cells (□). All other cells were from males with basal medium (●), basal medium plus 10 μg ml⁻¹ L-T₃ (X), or glucagon (○) or inosine (●), or insulin (◆) or hydrocortisone (▲) or inosine and insulin and hydrocortisone (×); complete medium minus serum (Δ). b, Growth curves (solid lines) of cells plated in complete medium ±0.4 mM L-arginine (■); without L-ornithine (●); or with ornithine replaced by 8 μM arginine (×) or by 0.008–0.4 mM putrescine (▲). Free arginine levels in culture media (except +0.4 mM arginine condition) are shown by dashed lines.

260 ng 'albumin' per 10⁵ cells per 6 h, respectively, whereas these rates measured by immunoprecipitation are 60,000 and 140,000 ³H-labelled 'albumin' c.p.m. per 10⁵ cells per 6 h. ³H-ornithine uptake is liver-specific because it is arginine-suppressible (Fig. 2) and detectable radioautographically as cytoplasmic grains⁶, mainly in monolayer aggregates⁴. Cells possessing this function (ornithine transcarbamylase) comprise 74 ± 3% (s.e.m.; N = 3) of the attached cell fraction 96–120 h post-plating. If log-phase cultures are labelled with both ³H-dT (as above) and ³H-ornithine (Fig. 2), radioautographic analyses show that 40 ± 2% (s.e.m.; N = 3) of ornithine-labelled cells also are in S-phase. Therefore, a substantial fraction of identifiable parenchymal hepatocytes traverse the 'cell-cycle' in this system. The extent to which DNA-synthesising cells not detectably labelled with ³H-ornithine (about 33% of the S-phase population) represent dedifferentiated hepatocytes and/or a contaminant population cross-fed via cell-cell interactions¹⁶ is as yet unknown.

Figure 2 also shows parallel increases in rates of ³H-ornithine uptake into cellular 'protein' and into released ³H-albumin during log growth; during stationary phase, these rates decrease to 30–40% and to about 80% of maximal, respectively. Moreover, arginine suppresses ³H-ornithine uptake into cellular 'protein' more efficiently than into released ³H-albumin. These observations could underlie intracellular compartmentalisation

of amino acid pools used for different protein synthetic processes in this system. This interpretation is complicated, however, by our inability rigorously to exclude the presence of heterogeneous cell types. Additional hepatic parenchymal cell functions present in this system include α_1 -foetoprotein, urea, tyrosine, and very low density lipoprotein biosynthesis (H. Leffert *et al.*, in preparation), and the hepatic microsomal hydroxylases¹⁷ (Fig. 3).

Conversion of ^{14}C -AAF into N -OH- ^{14}C -AAF (which is released into the medium) is shown in Fig. 3. This process appears liver cell-specific (e.g., primary adult rat pituitary or 3T3 cells lack this function) and is performed by viable cells, not by extracellular chemical and/or enzymatic mechanisms. Intracellular metabolism of N -OH- ^{14}C -AAF is suggested⁵ because cell-associated radioactivity, detected as 5% trichloroacetic acid-insoluble material, can be solubilised by trypsin, or KOH, or DNase treatment; moreover, unlabelled AAF specifically competes for ^{14}C -AAF uptake; and conversion is modified by gonadal steroids (H. Leffert *et al.*, in preparation). Relative proportions of hydroxylated metabolites released *in vitro* (N -OH-AAF = 2%, 3-, 5-, 7-, and 1-OH-AAF = 17, 15, 7, and 1%, respectively) simulate urinary metabolite distributions reported for male rats fed ^{14}C -AAF (ref. 18). This determination was independently confirmed using a second solvent system which, in addition to resolving N -OH- ^{14}C -AAF,

Fig. 2 Albumin production and ^3H -ornithine uptake in adult liver cell cultures. Male cultures were established using complete medium (see Fig. 1). Media were aspirated at various times, the dishes washed twice with 2 ml complete medium (minus ornithine) and then fed 2 ml similar medium containing 25 μCi ^3H -3-dl-ornithine (1 mCi per 68 μg ; New England Nuclear) without (open symbols) or with (shaded symbols) 0.4 mM arginine for 6–8 h. Harvested fluids were pretreated with an unrelated antigen-antibody system, to remove nonspecific radioactivity adventitiously adsorbed to specific immune-complexes, and then assayed for ^3H -labelled rat albumin ($\square$ — $\square$) by immunoprecipitation with purified monospecific goat anti-rat albumin antibody¹⁵. About 20% of the total radioactivity material present in the culture fluids was acid-insoluble; of this, 20–30% was detected as 'albumin' either by the above procedure or by isoelectric focusing together with authentic rat albumin¹⁵. Culture monolayers were assayed¹⁴ for precursor uptake into acid-insoluble material ($\circ$ — $\bullet$). A similar pattern is observed if uptake into 90 $^\circ\text{C}$ (20 min) heat-stable acid insoluble material is determined¹⁴; about 40% of the counts are solubilised at any time under these conditions. Measurement errors were $\pm 10\%$.

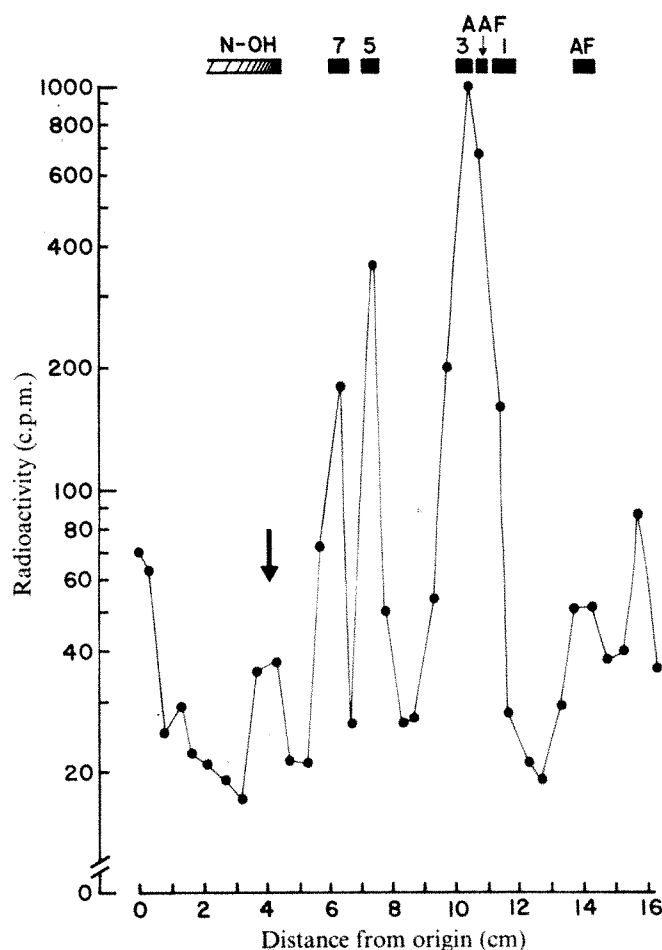
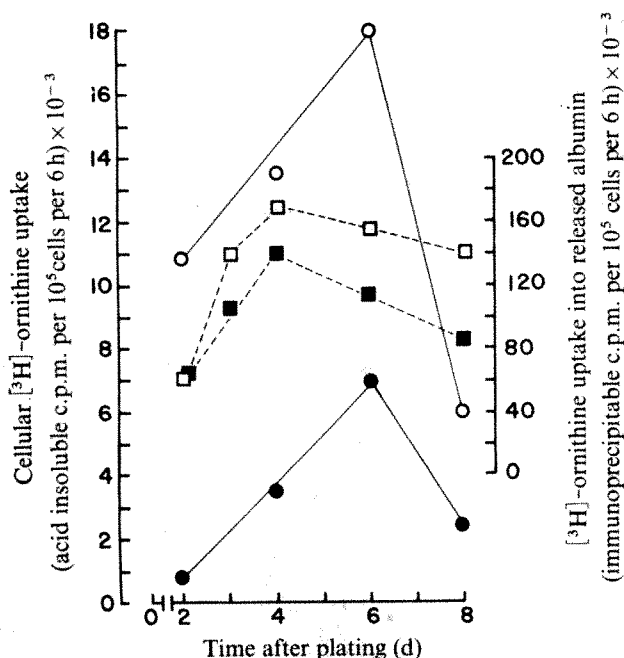


Fig. 3 N -hydroxylation of 2-acetylaminofluorene by cultured adult liver cells. Male cultures 5–6-d-old (Fig. 2) were treated 48 h with 20 μl of a 95% v/v ethanol solution of ^{14}C -9-AAF (0.1 mCi per 0.55 mg; 20 $\mu\text{Ci ml}^{-1}$; 100 $\mu\text{g ml}^{-1}$ (New England Nuclear); 96–98% of the radioactivity is found in the medium after 48 h. Two ml cell-free culture medium was added to 3 ml 1 M sodium acetate buffer, pH 6.0, containing 50 μl CHCl_3 and 2.5 mg each of β -glucuronidase and α -amylase (61 and 55 U mg^{-1} , respectively; Sigma)²⁰ and incubated for 24 h at 37 $^\circ\text{C}$. Acid-washed ether extracts of the resulting solution, which removed about 65% of the counts, were chromatographed^{19,20} on silica gel 60 plates 5×20 cm; Brinkman) using benzene/acetone (8/2) and developed four times; recovery was $\geq 98\%$. Radioactivity present in 5×0.5 cm strips scraped into plastic vials containing 10 ml 'cocktail' (3.79 l toluene, 160 ml Liquifluor, 158 g Cab-O-Sil (Eastman Kodak) was detected by scintillation counting with measurement errors of $\pm 0.2\%$. R_f values of authentic N -OH-AAF (cross-hatched rectangular bar), ring-hydroxylated metabolites, ^{14}C -AAF and aminofluorene (AF) (solid bars) are indicated above radioactive profile (corrected for background of 8 c.p.m.). Standards were detected with Folin/ Na_2CO_3 and p -nitro-methylbenzaldehyde reagents¹⁹. Peak fractions (arrow indicates position of N -OH- ^{14}C -AAF) were confirmed identical with authentic standards by rechromatography using a second system (chloroform/methanol, 97/3; development three times). Radioactivity in both systems which cochromatographed with authentic N -OH-AAF was five to eightfold greater than background.

separates 1- and 3-position ring-hydroxylated metabolites from AAF (see legend to Fig. 3)^{19,20}.

The critical interactions required of hormones, nutrients and carcinogens (at cellular and subcellular targets) to alter proliferative control are as yet unknown. This system may provide a useful tool to study this problem.

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Activation of human leukocyte elastase by human α_2 -macroglobulin

PROTEOLYSIS and its biological control in the pathogenesis of disease states involving inflammation and tissue injury have become areas of intensive study^{1,2}. Because of their probable involvement in the pathogenesis of emphysema, the elastolytic enzymes derived from neutrophils have received particular attention^{3–6}. Among the principal protease inhibitors in blood, α_1 -antitrypsin (α_1 -AT) serves as the main inhibitor controlling the activity of elastase and other serine proteinases, whereas α_2 -macroglobulin (α_2 -M) is believed to function primarily as a means of removing elastase and other proteinases from the circulatory system via the reticuloendothelial system^{7,8}. During studies of the interaction of various inhibitors with elastase isolated from purulent sputum⁹, it was noted that the activity of this enzyme towards certain synthetic substrates was markedly enhanced in the presence of α_2 -M. We present here a study of this activation. It is possible that activation of elastase by α_2 -M may be of physiological significance, particularly under those conditions where a deficiency of α_1 -antitrypsin is known to be a predisposing factor towards chronic obstructive pulmonary disease¹⁰.

Leukocytic elastase was prepared from purulent sputum⁹ and was comprised of three isozymes with no other impurities detectable by gel electrophoresis. For purposes of calculation, however, this mixture of enzymes was assumed to be a single entity with a molecular weight of 27,000 (ref. 9). human α_2 -M was prepared from plasma according to Harpel¹¹ and was judged to be at least 98% homogeneous by gel electrophoresis¹². Its molecular weight, as determined by sedimentation equilibrium, was 726,000. Although the activity of the elastase towards casein was almost completely inhibited by a twofold molar excess of α_2 -M, its activity towards the synthetic substrate Suc-(Ala)₃-Nan (*N*-succinyl-L-alanyl-L-alanyl-L-alanine-4-nitroanilide) was dramatically enhanced in the presence of α_2 -M (Fig. 1). Extrapolation of the linear segments of the curve in Fig. 1 shows maximum activation (15-fold) to occur when the weight ratio of α_2 -M to elastase is approximately 12 to 1. Based on the molecular weight values referred to above, this weight ratio corresponds to a molar ratio of 1:2.2. From Lineweaver–Burk plots, it was calculated that the k_{cat}

in the presence and absence of α_2 -M was 4.0 s^{-1} and 0.45 s^{-1} respectively, whereas the K_m was $1.82 \times 10^{-3}\text{ M}$ both in the presence and absence of α_2 -M. The fact that there was only a ninefold enhancement of the k_{cat} of elastase in the presence of α_2 -M compared to the 15-fold increase in the activity ratio (Fig. 1) may be due in part to the fact that in the latter case activity was based on end-point measurements at 37°C whereas the kinetic analysis was based on measurements of initial rates at 25°C .

Table 1 summarises data pertaining to the effect of α_2 -M on the activity of leukocyte elastase towards various other synthetic substrates. In addition, a comparison has been made with porcine pancreatic elastase (Worthington) in the case of Suc-(Ala)₃-Nan. The greatest degree of activation of the leukocytic elastase by human α_2 -M was observed with Suc-(Ala)₃-Nan as the substrate. Replacing the acidic succinyl group with an acetyl or a butoxy-carbonyl group produced a lesser degree of activation, and the substitution of the aromatic component of the ester linkage by a methyl group caused little, if any, activation. Of further interest is the fact that the activity of porcine pancreatic elastase towards Suc-(Ala)₃-Nan was actually inhibited by human α_2 -M.

Experiments were performed to ascertain whether this phenomenon of activation could be demonstrated with sera obtained

Fig. 1 Activation of human leukocytic elastase towards Suc-(Ala)₃-Nan by human α_2 -M. Reaction mixtures contained 100 μl of enzyme solution ($18\text{ }\mu\text{g ml}^{-1}$) with various levels (0–100 μl) of α_2 -M solution (1 mg ml^{-1}) in a final volume of 1.2 ml 0.2 M Tris-HCl–0.5 M NaCl, pH 8. After pre-incubation for 15 min at 37°C , 150 μl of substrate solution (0.01 M Sac-(Ala)₃-Nan; ref. 13) were added and the incubation continued for a further 20 min at the same temperature. Reaction was terminated by adding 150 μl glacial acetic acid, and the absorbance read at 410 nm. Insert is plot of the increase in absorbance (absorbance of enzyme with α_2 -M minus absorbance of enzyme without α_2 -M) as a function of the quantity of α_2 -M added. In the larger figure, the activity ratio (ratio of absorbance of enzyme with α_2 -M to absorbance of enzyme without α_2 -M) is plotted as a function of the ratio (by weight) of α_2 -M to enzyme.

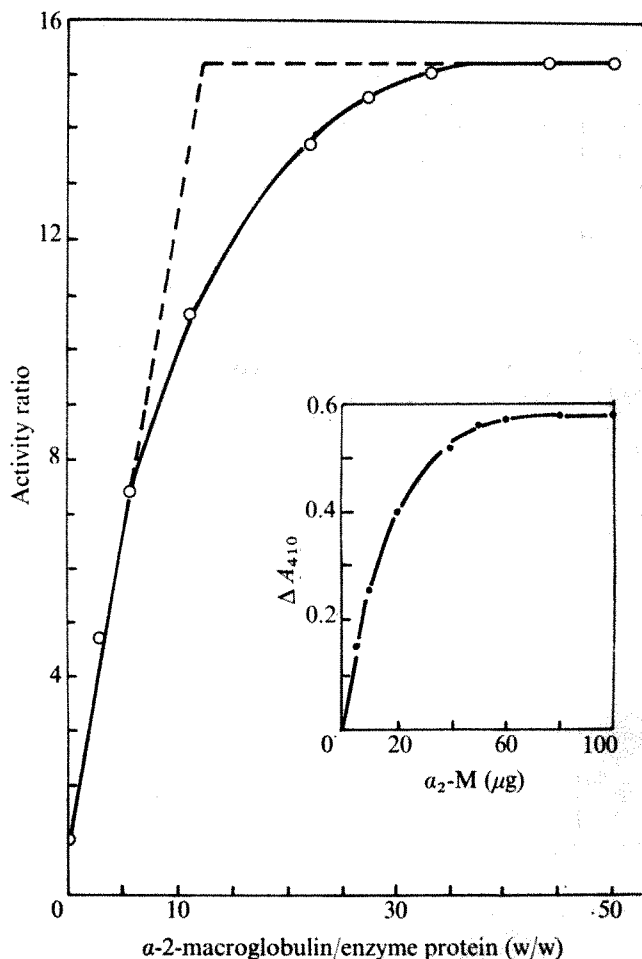


Table 1 Effect of human α_2 -M on the activity of elastases towards various synthetic substrates

Substrate*	Source of elastase	Specific activity†		Ratio + α_2 -M / - α_2 -M
		- α_2 -M	+ α_2 -M	
Suc-(Ala) ₃ -Nan	Leukocytes (human)	0.3	4.6	15.3
Suc-(Ala) ₃ -Nan	Pancreatic (porcine)	1.8	0.8	0.4
Ac-(Ala) ₃ -Nan	Leukocytes (human)	1.2	4.2	3.5
Boc-Ala-ONp	Leukocytes (human)	3.4	7.3	2.2
Ac-(Ala) ₃ -OMe	Leukocytes (human)	56.7	59.7	1.1

*Suc-(Ala)₃-Nan, *N*-succinyl-L-alanyl-L-alanyl-L-alanine-4-nitroanilide (assay in ref. 13); Ac-(Ala)₃-Nan, *N*-acetyl-L-alanyl-L-alanyl-L-alanine-4-nitroanilide (assay ref. 14); Boc-Ala-ONp, *N*-*t*-butoxycarbonyl-L-alanine-4-nitrophenylester (assay ref. 15); Ac-(Ala)₃-OMe, *N*-acetyl-L-alanyl-L-alanyl-L-alanine methyl ester (assay ref. 16).

†Units of activity per mg enzyme where one unit of activity is equivalent to the hydrolysis of 1 μ mol of substrate per min under the conditions specified for each assay.

from normal (phenotype *MM*) and α_1 -AT-deficient (phenotype *ZZ*) individuals. Data were obtained with sera from two patients who had been diagnosed as being α_1 -AT-deficient homozygotes (phenotype *ZZ*) and with sera from two normal individuals (phenotype *MM*) (Table 2). Both the normal and deficient sera completely inhibited the activity of the leukocyte and pancreatic elastases toward elastin. When the synthetic substrates were tested, the residual activity of both elastases was consistently higher in the case of the *ZZ* sera. Especially noteworthy, however, is the fact that there was a fourfold increase in the activity of the leukocyte elastase when tested against Suc-(Ala)₃-Nan in the presence of the *ZZ* sera. This observation can be readily explained by the fact that the predominant protease inhibitor in *ZZ* sera is α_2 -M rather than α_1 -AT, a situation which, on the basis of our previous experiments, would be expected to augment the activity of the leukocytic elastase towards Suc-(Ala)₃-Nan.

α_2 -M is known to interact with a wide variety of proteases to form complexes which are generally inactive towards large molecular weight substrates but which exhibit little or no loss in activity towards low molecular weight substrates¹⁷. Although there have been occasional reports of an enhancement of the activity of trypsin (bovine)¹⁸, chymotrypsin (bovine)¹⁹, and elastase (porcine)²⁰ by α_2 -M (human), the degree of activation has usually been quite small (10–50%) compared to that which we observed with our system (900–1,500%). We attribute this difference to two factors; first, both the enzyme and α_2 -M which we used were derived from the same species of animal; and second, the substrate used had an aromatic moiety as part of the ester or amide linkage and had an acidic group blocking the terminal amino group of the substrate. It is interesting to note that Nakamura *et al.*²¹ have also reported a two to threefold activation of bovine chymotrypsin by bovine α_2 -M on substrates possessing these same attributes. In agreement with the data of Nakamura *et al.*²¹ we also found that maximum activation of leukocyte elastase towards a synthetic substrate was produced when two molecules of the enzyme were complexed by one molecule of human α_2 -M. Ohlsson and Laurell⁸ have reported a similar ratio for the inactivation of

leukocyte elastase by human α_2 -M with elastin as the substrate.

At the moment we are unable to explain this phenomenon of activation, but any explanation that is offered should take into account the hypothesis that the complexation of proteases with α_2 -M is probably a two-step process involving an initial cleavage of a critical peptide bond followed by a conformational change which serves to entrap the enzyme¹⁷. Macromolecular substrates are thus prevented from reaching the enzyme, whereas small synthetic substrates are not so restricted. Particularly puzzling is the fact that the K_m , which reflects the affinity of the enzyme for the substrate, Suc-(Ala)₃-Nan, is not affected by α_2 -M despite a marked enhancement in the catalytic efficiency of the enzyme as measured by k_{cat} . Since the latter effect seems to depend on the presence of an aromatic and an acidic group on the substrate molecule, it may be that the conformational change in α_2 -M which serves to entrap the enzyme uncovers a hydrophobic cavity which, in conjunction with a positively charged group, orients the substrate in a position which is more favourable for acylation or deacylation by the enzyme. Jacquot-Armand and Krebs²² have in fact shown that α_2 -M which has interacted with trypsin undergoes a conformational change which exposes an apolar region in the α_2 -M molecule. Furthermore, it seems that the most striking effects are produced when the enzyme and α_2 -M are derived from the same species of animal—the reason for this remains obscure.

Whether this phenomenon of activation is of any physiological significance is open to conjecture. One observation which may be pertinent in this respect is the reference by Meyer *et al.*²³ to unpublished results showing that pancreatic elastase (porcine) was capable of digesting chemically solubilised elastin even when complexed with α_2 -M. If the leukocytic elastase were to behave in a comparable fashion, then its complexation with α_2 -M might actually serve to augment its ability to degrade tropoelastin secreted into the extracellular space prior to the formation of cross-linkages found in elastin²⁴. In normal individuals this destructive effect on the synthesis of elastin could be held in check by adequate levels of α_1 -AT. On the other hand, as suggested by our *in vitro* experiments with *ZZ* sera, in individuals who are deficient in α_1 -

Table 2 Effect of *MM* and *ZZ* sera on the activity of leukocyte and pancreatic elastases on various substrates *

Substrate†	Source of elastase	Phenotype‡			
		M.G.	<i>MM</i> P.L.	<i>ZZ</i> J.H.	N.G.
Elastin-rhodamine	Leukocytes (human)	0	0	0	0
	Pancreatic (porcine)	0	0	0	0
Suc-(Ala) ₃ -Nan	Leukocytes (human)	55	70	400	400
	Pancreatic (porcine)	8	13	40	45
Boc-Ala-ONp	Leukocytes (human)	10	—	50	48
	Pancreatic (porcine)	15	11	55	49

*Activities are expressed as % of controls without serum.

†Activity towards elastin-rhodamine was measured according to Rinderknecht *et al.*²⁵ using levels of 1.4 and 12.5 μ g of porcine and leukocyte elastase respectively in each assay mixture. Assays on synthetic substrates were performed as described in Table 1 using levels of 1.4 and 1.33 μ g of porcine and leukocyte elastase respectively in each assay system. The level of serum employed in these assays was 1.0 μ l serum per μ g enzyme in case of *MM* sera and 4.5 μ l serum per μ g enzyme for *ZZ* sera.

‡The trypsin inhibitor capacity of the serum of each individual was assayed on *N*-benzoyl-DL-arginine-*p*-nitroanilide²⁶, and the following values, expressed as % of a standard reference serum (supplied by Dr John A. Pierce) were obtained for the four individuals: M.G., 119; P.L., 100; J.H., 32; N.G., 25.

AT a greater proportion of the elastase would be expected to complex with α_2 -M leading to a further exacerbation of the destruction of lung tissue.

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Effects of iron chelators and iron overload on *Salmonella* infection

INCREASED susceptibility to infectious disease is observed in iron-overloaded states such as β -thalassaemia major and in haemolytic states such as sickle cell disease or Bartonellosis^{1,2}. The virulence of *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium* and other pathogens can be increased by administration of iron compounds to the host, and transferrin-induced inhibition of bacterial growth can be reversed by saturating transferrin^{3–6}. Plasma and secretory iron-binding proteins such as transferrin and lactoferrin may function in host defence by limiting the availability of iron to invading pathogens⁷. Many microorganisms synthesise iron-binding compounds when little iron is available⁸; these compounds may compete with host iron-binding mechanisms. We report here the effects of iron-chelating drugs on this competition for iron in *S. typhimurium* infection in normal and iron-overloaded mice. Desferrioxamine (DF), a hydroxamic acid, and 2,3-dihydroxybenzoic acid (2,3-DHB), a phenolic iron chelator, represent two classes of iron-chelating compounds which are being tested in chelation therapy for iron overload. Our data provide evidence that DF increases iron availability to wild type *S. typhimurium*, promoting bacterial growth and thus enhancing virulence.

Male Swiss-Webster mice were iron overloaded by intraperitoneal injections of 0.25 ml of human blood heated to 50 °C for 5 min. Injections were given once a day, five

times a week for 8 weeks, so that mice received more than 3 blood volumes before infection. After overloading, the liver, spleen and pancreas of several mice were examined histologically. Liver and spleen iron was also measured by atomic absorption spectrophotometry and compared with that of control mice. Iron overloading was confirmed in sections stained with Prussian blue, which revealed increased iron stores in the liver, spleen and pancreas. Concentrations of iron in normal mice were 159.4 ± 12.6 mg per liver and 44.6 ± 3.5 mg per spleen compared with 230.2 ± 8.8 mg per liver and 70.2 ± 8.8 mg per spleen in iron-overloaded animals.

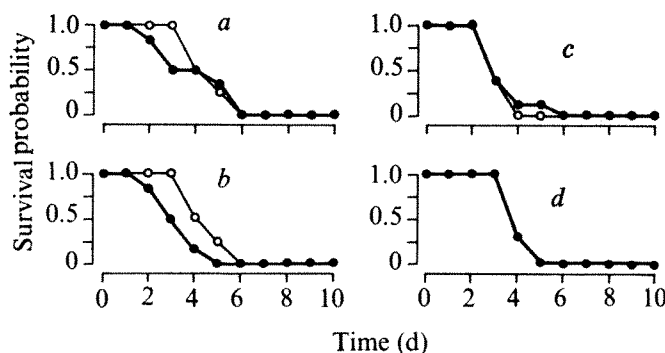
Wild type *S. typhimurium* was obtained from a clinical microbial laboratory and found to grow normally on low-iron medium. A mutant of *S. typhimurium* (TA2701) blocked in the synthesis of enterobactin, its own natural iron chelator, was unable to grow on low-iron media in the presence of DF but would grow in response to 2,3-DHB (refs 10, 11). After passage through six generations of mice to increase its virulence, the mutant retained its growth characteristics on low-iron medium. Organisms for injection were cultured overnight in nutrient broth, washed three times and diluted in sterile saline.

Iron-overloaded and normal mice received an intraperitoneal injection of either the wild type or mutant *S. typhimurium* and were divided into three groups of six. One group received DF, one received 2,3-DHB and the third received no drug. Both drugs were injected in doses of 25 mg kg⁻¹ every 6 h, beginning 24 h before infection and continuing throughout the experiment. Uninfected groups of six iron-overloaded or normal mice received DF or 2,3-DHB in the same doses. The number of survivors from each group was recorded at 6-h intervals.

Cultured post mortem liver specimens yielded organisms with the same growth characteristics on low-iron medium as the infecting organism. This confirms that the pathogen injected was the cause of death. All uninfected mice treated with 2,3-DHB or DF survived.

Figure 1 shows the effects of iron overloading on the survival probability of animals not receiving an iron-chelating drug. In the groups infected with wild type *S. typhimurium*, iron overloading caused a significant reduction in survival probability. Similarly, in the groups treated with DF and 2,3-DHB (not shown), iron overloading increased virulence in wild type infection, the effect being

Fig. 1 Survival probabilities against time of normal (○) and iron-overloaded (●) mice infected with *S. typhimurium*. a, Wild type, 10^8 bacteria, $P < 0.02$; b, wild type, 10^5 bacteria, $P < 0.02$; c, mutant TA2701, 10^4 bacteria, not significant; d, mutant TA2701, 10^6 bacteria, not significant. Probabilities were determined by the Stromgen method of life-table analysis¹². Differences in the probability plots were compared for significance at the maximum deviation using a two-sided hypothesis¹³.



most pronounced with DF. In untreated mice infected with the mutant (Fig. 1b), iron overloading had no effect on survival probabilities. Similarly, iron overloading produced no reduction in survival in DF-treated animals infected with the mutant. But in animals infected with the mutant and treated with 2,3-DHB, survival was reduced by iron overloading (Fig. 2).

Figure 3 shows that in wild type infection, survival of both normal and iron-overloaded animals was reduced significantly by DF. This effect was absent or reversed in animals infected with the mutant. The reversal of the DF effect—prolongation of survival—was seen in both normal and iron-overloaded animals given the lower infecting dose.

Wild type *S. typhimurium* which grow in low-iron media can do so, at least in part, because they synthesise an iron chelator such as enterobactin¹⁴. *In vivo*, if iron overloading produces an increase in host iron available to the pathogen, wild type growth rate and thus virulence would be expected to increase. This expectation was fulfilled in control and drug-treated animals which were iron overloaded and infected with the wild type *Salmonella*. If the ability to synthesise an iron chelator is absent, as in mutant TA2701, increased availability of iron in the host should have no effect on virulence. This is confirmed by Fig. 1b. The only mutant-infected animals to show reduced survival with iron overloading were those treated with 2,3-DHB. This was anticipated by the *in vitro* growth characteristics of TA2701, which can utilise 2,3-DHB as an iron chelator.

DF has been shown to increase the growth rate of *S. typhimurium* in defined medium¹⁰, and Fig. 3 confirms this effect *in vivo*. DF seems to increase the availability of iron to multiplying wild type *Salmonella* and thus to reduce the survival of the host. This effect is augmented by iron overloading. On the other hand, treatment of the mutant-infected animals with DF produced no significant increase in virulence. At the lower levels of infection in both normal and iron-overloaded animals, DF significantly prolonged survival. These results support the *in vitro* observation that the mutant cannot utilise ferrioxamine. Thus by sequestering iron and making it unavailable to the mutant, DF retards its growth and decreases its virulence.

These experiments provide evidence that DF, which is used to treat iron overload, may increase the virulence of *S. typhimurium* by increasing the iron available to the bacteria and thereby promoting growth. The results also suggest that an iron chelator not utilisable by micro-organisms decreases the iron available for bacterial growth

Fig. 2 Survival probabilities of normal (○) and iron-overloaded (●) mice treated with 2,3-DHB and infected with mutant TA2701. 2,3-DHB was synthesised in our laboratory⁹. a, 10^6 bacteria, $P < 0.02$; b, 10^4 bacteria, $P < 0.01$.

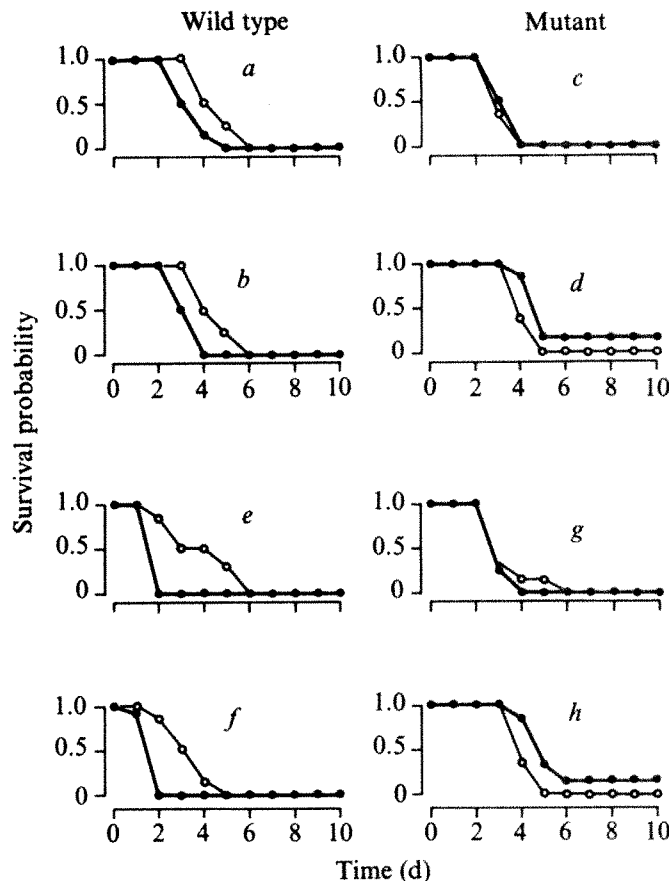
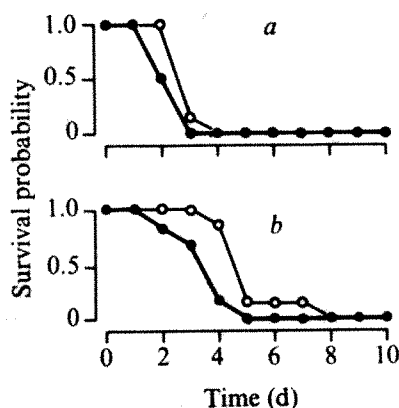


Fig. 3 The effect of DF (Ciba-Geigy) on the survival probabilities of normal (a-d) and iron-overloaded (e-h) mice infected with *S. typhimurium*. a, 10^6 bacteria, $P < 0.02$; b, 10^4 bacteria, $P < 0.02$; c, 10^6 bacteria, not significant; d, 10^4 bacteria, $P < 0.05$; e, 10^6 bacteria, $P < 0.001$; f, 10^4 bacteria, $P < 0.001$; g, 10^6 bacteria, not significant; h, 10^4 bacteria, $P < 0.05$. ○, Control (no drug); ●, treated with DF.

and thus decreases the virulence of invading pathogens.

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GABA receptor binding with $^3\text{H}(+)\text{bicuculline-methiodide}$ in rat CNS

NEUROTRANSMITTER receptors might be identified biochemically in binding studies with synaptic membrane preparations using labelled receptor agonists or antagonists as ligands¹. In attempts to identify the GABA receptor ^3H -GABA has been used as ligand. ^3H -GABA, however, also binds to glial and neuronal GABA uptake sites and to enzymes metabolising GABA. Thus, Na^+ -dependent ^3H -GABA-binding^{2,3}, in contrast to Na^+ -independent ^3H -GABA-binding is possibly not related to the GABA receptor⁴. In the present investigation the GABA antagonist $^3\text{H}(+)\text{bicuculline-methiodide}$ ($^3\text{H}(+)\text{BM}$) was used as ligand, which in contrast to bicuculline, is chemically sufficiently stable⁵. $(+)\text{Bicuculline}$ and its *N*-methyl-derivative antagonise reversibly and rather selectively the inhibitory synaptic action of GABA in the central nervous system (CNS) (refs 6, 7) without affecting the uptake or release of GABA or enzymes metabolising GABA (refs 8, 9). Thus $^3\text{H}(+)\text{BM}$ might be more suitable for GABA receptor binding studies than GABA itself.

The characteristics of specific $^3\text{H}(+)\text{BM}$ binding in the present study are compatible with the properties of the GABA receptor expected from neurophysiological data. Specific $^3\text{H}(+)\text{BM}$ -binding was saturable, stereospecific and maximally enriched in the synaptic membrane fraction. Among a variety of compounds tested only GABA, GABA agonists and GABA antagonists were potent competitors for $^3\text{H}(+)\text{BM}$ specific binding sites.

Routine binding assays were performed by incubating crude cerebellar synaptic membranes with 5 nM $^3\text{H}(+)\text{BM}$ in the absence and presence of exogenous GABA. 'Specific binding' of $^3\text{H}(+)\text{BM}$ to the membranes was obtained by subtracting from the radioactivity bound in the absence of GABA ('total binding') the amount not displaceable by high concentrations of GABA (50 μM ; 'non-specific binding').

Among the different subcellular fractions of cerebellum

tested, the highest specific $^3\text{H}(+)\text{BM}$ binding was found in the crude synaptic membrane fraction (Table 1). The specific activity (d.p.m. per mg protein) of $^3\text{H}(+)\text{BM}$ specifically bound in this fraction was about 2.5 times that of the whole homogenate and similar to that of the P_2 -fraction containing intact synaptosomes.

Specific $^3\text{H}(+)\text{BM}$ binding to the cerebellar synaptic membrane fraction was saturable, half-maximal binding occurring at 380 ± 20 nM. By contrast, non-specific binding, represented by the radioactivity bound in the presence of 50 μM GABA, was not saturable and increased linearly with increasing $^3\text{H}(+)\text{BM}$ concentration. Similarly, displacement of $^3\text{H}(+)\text{BM}$ by non-radioactive $(+)\text{BM}$ was half-maximal at 218 ± 21 nM (Table 2, Fig. 2), correlating well with the value obtained in the saturation experiments using $^3\text{H}(+)\text{BM}$ alone.

The stereospecificity of $^3\text{H}(+)\text{BM}$ binding is shown by the finding that the neurophysiologically inactive isomers $(-)\text{bicuculline}$ and $(-)\text{BM}$ (ref. 10) had only about 1/800 and 1/400 the affinity of the respective $(+)\text{isomers}$ for the $^3\text{H}(+)\text{BM}$ binding sites, as indicated by their K_i values (Table 2, Fig. 1). Furthermore, when $^3\text{H}(-)\text{BM}$ was used as ligand (5 nM), no displacement with 50 μM non-radioactive $(+)\text{BM}$, GABA, muscimol, 3-aminopropane-sulphonic acid or $(-)\text{BM}$ was observed, indicating that the neurophysiologically inactive isomer $(-)\text{BM}$ did not bind to the $(+)\text{BM}$ binding sites at the ligand concentration used. In addition, since the excitatory action of $(+)\text{BM}$ found *in vivo* is not stereospecific¹⁰, this action does not seem to be reflected in the *in vitro* $^3\text{H}(+)\text{BM}$ binding studies presented here.

The high affinity of $(+)\text{bicuculline}$ ($K_i = 68$ nM) for the $(+)\text{BM}$ binding sites (Table 2, Fig. 1) corresponds to its high potency as a GABA antagonist *in vivo*; $(+)\text{bicuculline}$ is about 60 times less potent in competing for Na^+ -independent ^3H -GABA binding sites (rat whole brain⁴) and even 1,000 times (rat cerebellar cortex³) and 70,000 times (crayfish muscle²) less potent in competing for Na^+ -dependent ^3H -GABA binding sites.

The findings that $(+)\text{BM}$ is more potent than $(+)\text{bicuculline}$ as a GABA antagonist microelectrophoretically⁷ and as a convulsant¹¹ are difficult to explain on the basis of their potencies in competing for $^3\text{H}(+)\text{BM}$ binding sites (Table 2, Fig. 1). However, the high lipid solubility of bicuculline might influence its concentration at the receptor or binding sites to a varying degree under different experimental conditions. In addition, there might be a

Table 1 Subcellular distribution of specifically bound $^3\text{H}(+)\text{BM}$ in rat cerebellum

Fraction	Specifically bound $^3\text{H}(+)\text{BM}$ (d.p.m. per mg protein)	(as % of total binding)	Specifically bound $^3\text{H}(+)\text{BM}$ per entire fraction (d.p.m.)
Whole homogenate	471 $\pm$ 34	16.7 $\pm$ 1.0	225,200 $\pm$ 10,400
Crude nuclear pellet (P_1)	318 $\pm$ 97	13.5 $\pm$ 3.3	85,600 $\pm$ 25,400
Crude mitochondrial pellet (P_2)	1,082 $\pm$ 17	28.7 $\pm$ 0.5	128,100 $\pm$ 6,500
Crude microsomal pellet (P_3)	204 $\pm$ 14	8.9 $\pm$ 0.8	11,390 $\pm$ 1,170
P_2 subfractions after osmotic shock:			
Mitochondria-myeelin pellet	422 $\pm$ 7	14.9 $\pm$ 0.2	7,900 $\pm$ 1,050
Crude synaptic membrane pellet	1,044 $\pm$ 34	28.7 $\pm$ 0.7	116,600 $\pm$ 5,100

The subcellular fractions of cerebellum (male SPF rats, 150-200 g) were prepared according to Zukin *et al.*⁵. The binding assay was performed in triplicate as described in Fig. 1 (5 nM $^3\text{H}(+)\text{BM}$ and 2 mg protein per assay in the presence or absence of 50 (μM) GABA). 'Total binding' refers to the radioactivity bound in the absence of GABA, 'specifically bound' to that displaceable by GABA. The values are the means $\pm$ s.e.m. from three experiments.

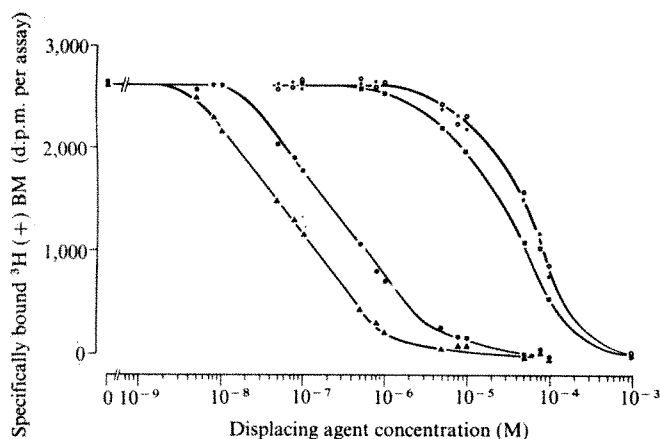


Fig. 1 Competition for the $^3\text{H}(+)\text{BM}$ specific binding sites by (+) bicuculline (▲); (—) bicuculline (*) (●); (+) bicuculline methiodide (○); (—) bicuculline methiodide (○); (±) laudanosine (■). Increasing concentrations of the displacing agent were added to tubes containing in a 2 ml volume cerebellar synaptic membranes (2 mg protein), 5 nM $^3\text{H}(+)\text{BM}$, 50 mM Tris-HCl buffer pH 7.4 and 50 mM NaSCN. After incubation at 25 °C for 15 min—at which time equilibrium had been reached—and centrifugation at 48,000 g for 10 min, the pellet was rinsed by superfusion with 5 ml and then 10 ml of ice-cold 50 mM Tris-HCl buffer pH 7.4. After dissolving the pellet in 2 ml Protosol, scintillant was added and the radioactivity counted (counting efficiency 38%). The points are the means of triplicate determinations of a typical experiment with standard errors of the mean ranging from 0.1 to 2.0%. The corresponding K_i values are given in Table 2. $^3\text{H}(+)\text{BM}$ and $^3\text{H}(-)\text{BM}$ (radiochemically pure > 99%; specific activity 8.0 and 13.0 Ci per mmol respectively) were prepared by methylation¹⁵ of (+) and (—) bicuculline with ^3H -labelled methyl iodide synthesised according to Schwob and Würsch¹⁶. (—) Bicuculline was synthesised according to Teitel *et al.*¹⁷. Crude synaptic membrane fractions were prepared according to Enna and Snyder⁴ and stored frozen at -30 °C for 1–5 d before use. Fresh and frozen membranes gave similar results. There was no degradation of bound or free $^3\text{H}(+)\text{BM}$ during the incubation.

contribution from the excitatory effect of (+)BM *in vivo* or a regional or species difference in the potency of the two compounds.

In agreement with the neurophysiological potency⁶, the GABA agonist muscimol was more, imidazole acetic acid less potent in competing for $^3\text{H}(+)\text{BM}$ binding sites than GABA itself, while 3-aminopropanesulphonic acid despite

Fig. 2 Competition for the $^3\text{H}(+)\text{BM}$ specific binding sites by GABA and GABA agonists. The binding assay was performed as described in Fig. 1 (5 nM $^3\text{H}(+)\text{BM}$; 2 mg cerebellar synaptic membrane protein per assay). Points are means of triplicate determinations in a typical experiment with s.e.m. values of 0.1–2.5%. The corresponding K_i -values are given in Table 2. Muscimol (▲); 3-aminopropanesulphonic acid (●); GABA (*) (○); imidazole acetic acid (■).

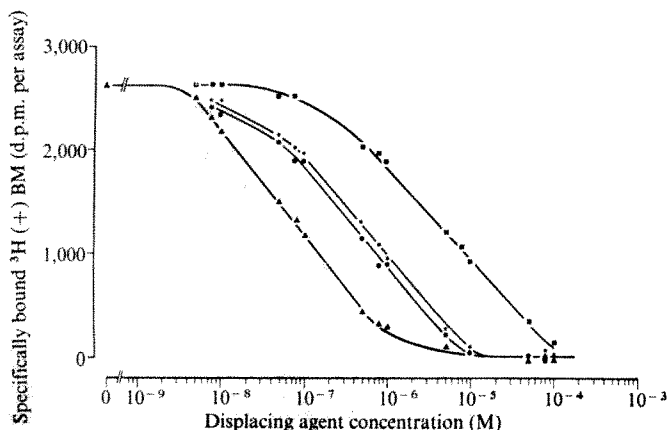


Table 2 Inhibition of specific $^3\text{H}(+)\text{BM}$ -binding to synaptic membranes of rat cerebellum by various compounds

Compound	K_i (nM)
GABA antagonists	
(+) Bicuculline	68.2 ± 2.5
(+) Bicuculline-methiodide	218 ± 21
GABA agonists, amino acids and related compounds	
Muscimol	58.1 ± 6.8
GABA	420 ± 55
3-Aminopropanesulphonic acid	490 ± 70
Imidazole acetic acid	3,830 ± 320
β-Alanine	58,700 ± 6,300
Glutamate	n.i.
Aspartate	n.i.
α-Alanine	n.i.
Glycine	n.i.
Proline	n.i.
Taurine	n.i.
Nipecotic acid	n.i.
Aminooxyacetic acid	n.i.
Isoniazid	n.i.
Other compounds	
Strychnine	3,500 ± 360
(±) Laudanosine	33,700 ± 1,600
(—) Bicuculline-methiodide	52,800 ± 5,200
(—) Bicuculline	59,300 ± 3,500

'No inhibition' (n.i.) refers to concentrations up to 10^{-4} M. Further compounds without inhibitory effect at least up to 10^{-8} M include 2,4-diaminobutyrate, morphine, nialamide, kainic acid, chlordiazepoxide, diazepam, pentobarbital, baclofen, oxotremorine, physostigmine and Ro 4-1284, a water-soluble benzoquinolizidine derivative with reserpine-like action¹⁸. Haloperidol and chlorpromazine displaced 18% of the specifically bound $^3\text{H}(+)\text{BM}$ at 10^{-5} M. Inhibition of $^3\text{H}(+)\text{BM}$ -binding (5 nM; 2 mg cerebellar membrane protein per assay) was measured at six to 15 concentrations of each compound in triplicate as described in Fig. 1. The 50% inhibitory concentration (IC_{50}) was calculated by log logit analysis and converted to K_i according to the equation $K_i = \text{IC}_{50}/(1 + C/K_D)$, where C is the concentration of radioactive ligand and K_D its dissociation constant. The values are the means ± s.e.m. of three experiments. For some compounds typical inhibition curves are shown in Figs 1 and 2.

its higher neurophysiological potency¹² was similar to GABA in its competing potency (Table 2, Fig. 2). As drug potencies can differ in different species and/or brain areas, however (as shown for muscimol and bicuculline⁶), a more quantitative correlation has to await binding experiments and neurophysiological studies to be performed in the same species and brain region.

It should be noted that (±)laudanosine, a neurophysiologically inactive analogue of bicuculline^{6,7}, is also inactive as inhibitor of specific $^3\text{H}(+)\text{BM}$ binding (Table 2, Fig. 1). Strychnine, with an affinity constant for the glycine receptor of 2 nM (ref. 13), required concentrations 1,700 times higher to reduce specific $^3\text{H}(+)\text{BM}$ binding by 50%.

In studies of the distribution of specifically bound $^3\text{H}(+)\text{BM}$ in different brain regions the highest levels were found in cerebral cortex (82.7 ± 1.4 fmol per mg protein) and cerebellum (67.6 ± 1.2), followed by hippocampus (57.1 ± 1.5), midbrain (54.2 ± 2.5), hypothalamus (49.4 ± 2.1) and striatum (39.2 ± 1.9). The lowest levels were found in medulla oblongata-pons (16.1 ± 1.7) and spinal cord (8.7 ± 2.0). These regional differences do not correlate well with the regional levels of endogenous GABA in rat CNS¹⁴. In other receptor systems, however, there are also discrepancies between receptor densities and endogenous transmitter levels. Presumably, the surface area and receptor density of the postsynaptic membrane vary independently of the endogenous transmitter levels in different areas of brain¹. The regional distribution of specific $^3\text{H}(+)\text{BM}$ -binding is largely similar to that of Na-independent ^3H -GABA-binding with the cerebellum as main exception which shows twice the GABA-binding of cerebral cortex⁴.

The GABA agonists tested so far in Na⁺-independent

³H-GABA-binding⁴ and in ³H(+)-BM-binding (GABA, 3-aminopropanesulphonic acid, imidazole acetic acid) show a higher affinity for ³H-GABA binding sites than for ³H(+)-BM binding sites. The inverse relationship holds for (+)-bicuculline, the only GABA antagonist compared so far. This finding may be explained by a receptor model in which agonists and antagonists bind to two different receptor sites possibly with a mutual negative cooperative interaction. Binding of an agonist might reduce the binding affinity of an antagonist and vice versa. But as the rank orders of regional ³H-GABA-binding and ³H(+)-BM-binding are not identical, we cannot exclude the possibility that ³H-GABA and ³H(+)-BM label different types of GABA receptors at least in some rat brain areas.

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Direct detection of depolarisation-induced release of ATP from a synaptosomal preparation

SEVERAL groups have shown that ATP is released from neural tissues during depolarisation, where it or its metabolites might function as neurotransmitters or neuromodulators¹. They measured radiolabelled adenosine derivatives in perfusates following depolarisation of their preparations. Recently, Israel *et al.*² described a method for continuously and directly detecting the release of ATP from the electric organ of *Torpedo marmorata* after stimulation of its motor nerve. I have used a modification of this technique to study the depolarisation-induced release of ATP from a purified synaptosomal fraction prepared from rat brain. I report here that depolarisation of synaptosomes elicits a release of ATP which can be directly and continuously detected by this technique.

Synaptosomes were incubated in a Krebs-Henseleit bicarbonate medium containing firefly luciferin-luciferase and depolarised by elevating the extracellular K⁺ or by adding veratridine. Veratridine depolarises excitable tissues, including synaptosomes, by selectively opening Na⁺ channels³⁻⁶. Released ATP was detected by measuring the light emitted with a photomultiplier. Supplementing the luciferin⁷ greatly enhanced the sensitivity of detection of ATP. Preliminary experiments showed that the assay system was specific for ATP, in that similar concentrations of adenosine, AMP or ADP did not produce chemiluminescence.

Depolarisation of synaptosomes caused a release of ATP

which could be directly and continuously monitored. Elevating the extracellular KCl concentration by 23 mM caused a rapid release of ATP from synaptosomes, indicated by an increased emission of light (Fig. 1a). This effect was probably not due to the increased tonicity of the incubation medium, since increasing the extracellular concentration of NaCl by 23 mM caused a fall in the background chemiluminescence. Choline chloride and LiCl produced similar decreases in chemiluminescence (not shown). The response to elevated extracellular K⁺ was not prevented by the specific Na⁺ channel blocker, tetrodotoxin⁸⁻¹⁰ (Fig. 1b), showing that this effect was not mediated by the opening of Na⁺ channels in the synaptosomal membranes. This is not surprising, since one would expect elevated extracellular K⁺ to depolarise the synaptosomes directly without altering the Na⁺ and K⁺ permeabilities^{11,12}.

The depolarisation-induced release of a number of putative neurotransmitters from brain preparations has been

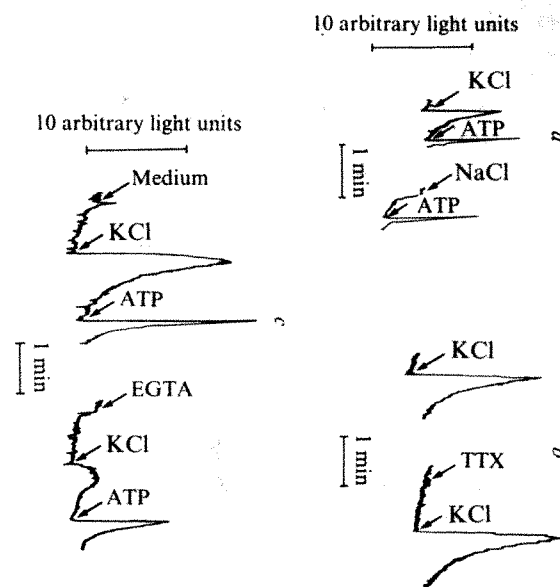


Fig. 1 K⁺-induced release of ATP from synaptosomes. Synaptosomes were prepared from the whole brains of four adult male Wistar rats (200-250 g) and isolated on sucrose density gradients as described previously⁸. They were finally suspended to a final volume of 8 ml with Krebs-Henseleit bicarbonate medium containing 111 mM NaCl, 26.2 mM NaHCO₃, 1.2 mM NaH₂PO₄, 4.7 mM KCl, 1.8 mM CaCl₂, 1.2 mM MgCl₂, 11 mM glucose and gassed periodically with 95% O₂: 5% CO₂ to maintain a pH of 7.4. The suspension was preincubated at 37 °C for 30 min and a 0.5 ml portion placed in a 6 × 50 mm cylindrical cuvette; 20 µl of fortified luciferin-luciferase enzyme preparation were added. The enzyme preparation was prepared daily by adding 0.2 ml synthetic D-luciferin (5 mg per ml H₂O, Sigma; stored at -20 °C) to 0.2 ml of crude firefly lantern extract (50 mg Sigma FLE-50 in 2 ml H₂O, prepared daily). The cuvette was placed in the reaction chamber of a 'Chem-Glow' chemiluminometer (American Instrument Co.) and let stand until a reasonably constant level of chemiluminescence was attained, usually 1-2 min. Even without gassing, the pH of the suspension remained at 7.5-7.6 throughout the incubation. *a*, KCl was increased by 23 mM by injecting 10 µl of a 1.25 M KCl solution into the cuvette with a Hamilton syringe. After the chemiluminescence had returned to its resting level, 5 µl of a 10⁻⁷ M ATP solution in incubation medium were injected into the cuvette to give a final ATP concentration of 9.4 × 10⁻¹⁰ M. The experiment was repeated using NaCl instead of KCl. *b*, Injecting 10 µl of 2.2 × 10⁻⁴ M tetrodotoxin (TTX) dissolved in incubation medium into the cuvette (Calbiochem, final concentration 4 × 10⁻⁷ M) did not prevent the release of ATP by elevated extracellular K⁺ (increased by 47 mM). *c*, To chelate Ca²⁺, 10 µl of 250 mM EGTA (pH 7.4 with NaOH) were injected into the cuvette (final concentration of 4.7 mM EGTA). Removal of Ca²⁺ by EGTA reduced the maximal response to added ATP to about 57% of the control value but reduced the maximal response to increased extracellular KCl to about 21% of the control value.

shown to be Ca^{2+} -dependent¹³⁻¹⁶. In the present study, the presence of 4.7 mM EGTA to chelate Ca^{2+} seemed to reduce the release of ATP by elevated extracellular K^+ (Fig. 1c), but EGTA also reduced the sensitivity of the luciferin-luciferase system to added ATP. Closer examination shows, however, that EGTA reduced the maximum K^+ response to about 21% of the control value, whereas EGTA only reduced the sensitivity of the system to added ATP to about 57% of the control. It therefore seems that the release of ATP from synaptosomes by elevated extracellular K^+ is at least in part Ca^{2+} -dependent.

Injection of veratridine into a synaptosomal suspension caused a rapid release of ATP which could be prevented by the prior administration of tetrodotoxin (Fig. 2). Tetrodotoxin added at the peak of the veratridine response caused an abrupt fall in the observed release of ATP. These findings indicate that the release of ATP by veratridine, in contrast to that caused by elevated K^+ (see Fig. 1), was mediated by the opening of Na^+ channels. Surprisingly, veratridine produced a more prolonged release of ATP than did elevated K^+ . The nature of this prolonged release of ATP is currently under investigation.

This study clearly demonstrates a depolarisation-induced and possibly Ca^{2+} -dependent release of ATP from synaptosomal preparations. As to the origin of the ATP

released, it seems most likely that presynaptic nerve-terminals are involved, although it is also possible that glial contaminants of the synaptosomal preparation¹⁷ might respond to depolarising influences by releasing ATP. Elevated extracellular K^+ has been reported to release γ -aminobutyric acid from glial cells in dorsal root ganglia by a Ca^{2+} -dependent mechanism¹⁸. On the other hand, the veratridine-induced release of ATP from synaptosomal preparations indicates an involvement of Na^+ channels, which glial elements may not possess.

Whether or not the ATP is released in its own right or in conjunction with the release of neurotransmitters¹³⁻¹⁶ is not known. In either case, it does not seem reasonable that such an energetically valuable molecule as ATP would be released and hydrolysed simply to provide a source of adenosine to promote the post-synaptic synthesis of cyclic-3', 5' AMP (ref. 1). Perhaps the extracellular hydrolysis of released ATP provides energy for some functionally important process in the synaptic region.

Numerous other questions arise as to the nature, distribution and role of the ATP release process in brain. This new method for directly studying the depolarisation-induced release of ATP from synaptosomes may assist in answering these questions.

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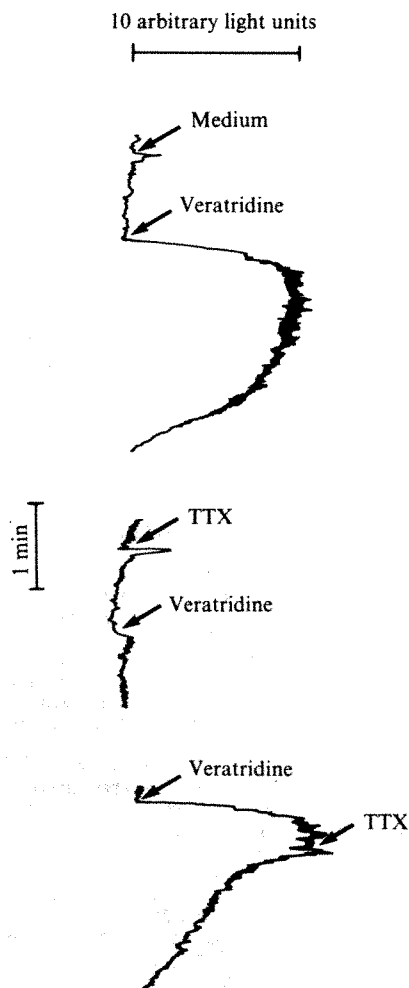


Fig. 2 Effect of veratridine on the release of ATP from synaptosomes. Synaptosomes were prepared and incubated as for Fig. 1. A stock solution of 27 mM veratridine (Aldrich) in ethanol was diluted 1:10 with incubation medium and 10 μ l of the latter solution were injected into the cuvette, giving a final concentration of 5×10^{-6} M veratridine. TTX in incubation medium was injected to give a final concentration of 4×10^{-7} M. Control injections of equivalent concentrations of ethanol did not promote chemiluminescence and a preliminary study showed that neither veratridine nor tetrodotoxin interfered with the response of the luciferin-luciferase system to added ATP.

Role of electrogenic sodium pump in slow synaptic inhibition is re-evaluated

THE mechanism of synaptic transmission at most synapses involves an increase in conductance of the postsynaptic membrane to certain ions^{1,2}. Certain slow postsynaptic inhibitions in both the central and peripheral nervous systems are, however, not generated in this way³⁻¹³. Nishi and Koketsu³⁻⁵ proposed that the slow inhibitory postsynaptic potential (IPSP) in bullfrog sympathetic ganglia is due to a unique mechanism, the synaptic activation of the electrogenic Na pump (see also refs 11-15). In many, if not all, neurones, the outward pumping of sodium is coupled to the inward transport of K, with more Na extruded than K taken in, thus producing an outward movement of positive charge¹⁶. This electrogenic pumping of Na, which is blocked by ouabain or K-free Ringer, produces a hyperpolarisation of the membrane without a change in membrane permeability¹⁶. In the present experiments, the direct role of the electrogenic Na pump in slow synaptic inhibition was tested. The experiments were carried out on the ninth or tenth paravertebral sympathetic ganglion of the bullfrog using the sucrose gap technique. We found that although electrogenic Na pumping was inhibited by

ouabain or K-free Ringer, the slow IPSP was not blocked by ouabain and was enhanced by K-free Ringer. The data are consistent with the hypothesis that the slow IPSP may result from an inactivation of Na conductance rather than from an activation of the electrogenic Na pump.

In mammalian sympathetic ganglia, the administration of nicotinic cholinergic agonists produces a brief depolarisation due to an increased conductance to Na^+ and K^+ . This is followed by an after-hyperpolarisation that results from the electrogenic extrusion of accumulated intracellular Na^+ ions¹⁷⁻¹⁹. We used this nicotinic after-hyperpolarisation to characterise the inhibition of electrogenic Na pumping in frog sympathetic ganglia by ouabain and K-free Ringer. Figure 1a (middle) shows that K-free Ringer rapidly inhibits the after-hyperpolarisation. This inhibition persisted as long as the preparation was bathed in K-free Ringer, but was rapidly reversible (Fig. 1a, bottom). Inhibition of the after-hyperpolarisation by ouabain occurred more slowly. Figure 1b (middle) shows that the after-hyperpolarisation following an acetylcholine (ACh) depolarisation was abolished after 1 h in ouabain 10^{-6} M. This effect was only slowly reversible, with the restoration of the ACh after-hyperpolarisation

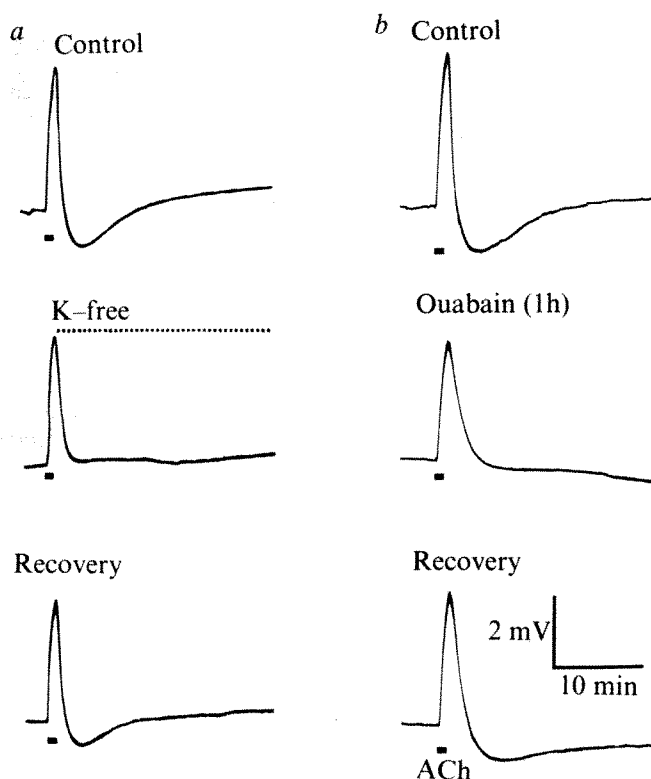


Fig. 1 Effects of K-free Ringer and ouabain on electrogenic sodium pumping. *a*, Effect of K-free Ringer: *top*, control response of sympathetic ganglion to administration of ACh (3 mM) for 1 min (bar) (note rapid depolarisation due to increased Na and K conductance followed by more prolonged after-hyperpolarisation resulting from electrogenic pumping of Na); *middle*, response when superfusion with K-free Ringer (dotted line) started at peak of ACh depolarisation (note block of after-hyperpolarisation); *bottom*, recovery of the after-hyperpolarisation following an ACh depolarisation (recorded 25 min after return to normal Ringer solution). *b*, Effect of ouabain: *top*, control response to administration of ACh (2.5 mM) for 1 min (bar) (as in *a*, but from a different preparation); *middle*, response to ACh after superfusion for 1 h with Ringer solution containing ouabain (10^{-6} M) (note block of the after-hyperpolarisation); *bottom*, recovery of the after-hyperpolarisation following an ACh depolarisation, after washing with normal Ringer's solution for 4 h 45 min. All records from a rectilinear pen recorder (Brush Model 280). Experiments were carried out on the ninth or tenth paravertebral sympathetic ganglion of the bullfrog using sucrose gap recording⁴. The preparation was continuously perfused with oxygenated Ringer solution of the following composition (mM): NaCl 100, KCl 2, CaCl_2 1.8, Tris HCl 16, pH 7.2, and glucose 1 g l^{-1} . K-free Ringer had the same composition except that KCl was omitted.

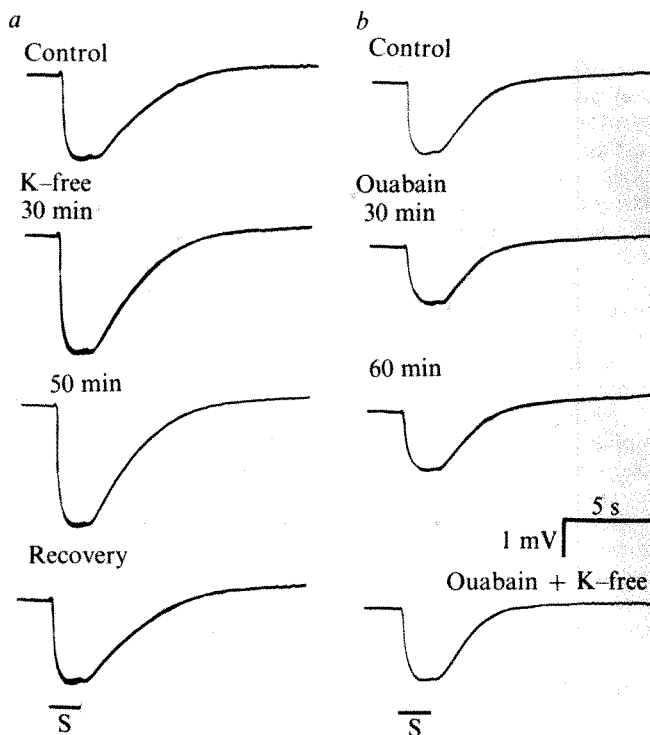


Fig. 2 Effects of K-free Ringer and ouabain on the slow IPSP. *a*, Effect of K-free Ringer: *top*, control record of slow IPSP elicited by repetitive stimulation of the eighth spinal nerve with 1-ms pulses at a frequency of 50 Hz (2-s period of stimulation is indicated by the line labelled S under bottom record; nicotine $30 \mu\text{M}$ used throughout the experiment to antagonise the fast EPSP); *middle*, slow IPSPs recorded 30 and 50 min after beginning superfusion of ganglion with K-free Ringer (note increase in slow IPSP amplitude in K-free Ringer); *bottom*, recovery of slow IPSP recorded 25 min after return to normal Ringer; *b*, Effect of ouabain: *top*, control record of slow IPSP (as in *a* except from a different preparation; nicotine ($30 \mu\text{M}$) was used throughout the experiment to antagonise the fast EPSP); *middle*, slow IPSPs recorded 30 and 60 min after beginning superfusion with Ringer solution containing ouabain (10^{-6} M) (note small reduction in slow IPSP amplitude compared with control); *bottom*, slow IPSP after 20 min superfusion with K-free Ringer containing ouabain (10^{-6} M) (record taken after 90 min in ouabain (10^{-6} M), with 30 and 60 min records shown above; note increase in slow IPSP amplitude compared with 30- and 60-min records). Records from photographs of oscilloscope traces.

taking 4 h or more (Fig. 1b, bottom). These results indicate that K-free Ringer and ouabain are effective inhibitors of the electrogenic pumping of Na in the bullfrog sympathetic ganglion. We therefore used these agents to test the hypothesis that the slow IPSP is generated by the activation of the electrogenic Na pump.

Figure 2 illustrates the effects of K-free Ringer and ouabain on the slow IPSP. The application of K-free Ringer solution produced little or no change in resting membrane potential, but resulted in a rapid and persistent increase in the amplitude of the slow IPSP. Figure 2a illustrates the increase in slow IPSP amplitude after 30 and 50 min in K-free Ringer. To investigate the muscarinic slow IPSP, the nicotinic fast excitatory postsynaptic potential (EPSP) must be antagonised. When nicotine ($30 \mu\text{M}$) was used to antagonise the fast EPSP, 1 h in ouabain (10^{-6} M) resulted in a partial (10–40%) reduction in slow IPSP amplitude (Fig. 2b). When d-tubocurarine ($70 \mu\text{M}$) was used as the nicotinic antagonist, however, no significant reduction was observed after 1 h in ouabain (10^{-6} M). Figure 2b (bottom) shows that even after 90 min in ouabain, the administration of K-free Ringer still increased the amplitude of the slow IPSP.

Two hypotheses have been advanced to explain the electrogenesis of the slow IPSP. One proposes that the

slow IPSP is due to the activation of the electrogenic Na pump³⁻⁵. The present experiments show that although K-free Ringer and ouabain inhibit the electrogenic pumping of Na, they do not block the slow IPSP. The other hypothesis proposes that the slow IPSP is generated by a decrease in resting Na conductance⁷. This mechanism would readily explain the effects of ouabain and K-free Ringer in the present experiments. When the fast EPSP was antagonised by nicotine, the slow IPSP was partially reduced in amplitude after 1 h in ouabain. It is known that nicotine can increase resting Na conductance²⁰, and in the presence of ouabain, which inhibits the extrusion of Na⁺, an accumulation of intracellular Na would be expected¹⁹. This accumulation would decrease the Na gradient and thus reduce the amplitude of a slow IPSP generated by a decrease in resting sodium conductance^{2,7}. This interpretation is supported by the observation that when *d*-tubocurarine, a nicotinic antagonist that does not increase resting Na conductance²¹, was used, ouabain did not produce a significant reduction in slow IPSP amplitude. The enhancement of slow IPSP amplitude by K-free Ringer is difficult, if not impossible, to explain in terms of the electrogenic Na pump hypothesis, since the presence of extracellular K⁺ is necessary for the coupled extrusion of Na⁺ (ref. 16). On the other hand, such an enhancement is predicted by the inactivation of Na conductance hypothesis because the driving force for the slow IPSP is the K equilibrium potential, E_K (ref. 7), which would be increased in K-free Ringer, thus increasing the amplitude of the slow IPSP. Furthermore, if the slow IPSP were due to electrogenic Na pumping, the combination of ouabain and K-free Ringer would be expected to result in a greater reduction in slow IPSP amplitude. The observed enhancement (Fig. 2b), however, can be explained by the Na inactivation hypothesis, as discussed above. The present experiments suggest that the slow IPSP is not due to the synaptic activation of the electrogenic Na pump. On the other hand, the results can be readily explained by and support the hypothesis that the slow IPSP is generated by an inactivation of resting Na conductance.

The electrophysiological properties of the slow IPSP (ref. 7) are remarkably similar to both the hyperpolarising response of vertebrate photoreceptors to light²²⁻²⁴ and central neurones to noradrenaline⁸⁻¹⁰. The conductance decrease mechanism may therefore be of general significance in understanding the generation of slow inhibitions in the nervous system. Furthermore, this mechanism may have important functional consequences. In central neurones, such a hyperpolarisation of the membrane would inhibit neuronal discharge; the concomitant increase in membrane resistance, however, might be expected to increase the amplitude of convergent EPSPs (refs 25 and 26). This could result in an improved signal to noise ratio for convergent synaptic excitation.

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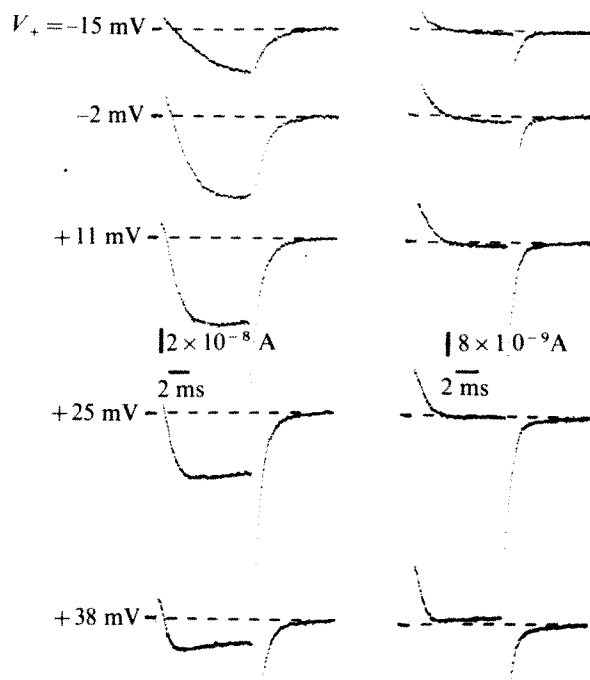
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Asymmetrical displacement currents in nerve cell membrane and effect of internal fluoride

WE have recorded asymmetrical displacement currents which may be linked to the activation of calcium permeability in the membrane of isolated snail neurones. The search for these currents was prompted by two lines of evidence. First, the existence of 'gating current' associated with sodium channel activity¹. And second, the existence of voltage-dependent charge movement in muscle fibres, which has some role in excitation-contraction coupling² and is supposedly involved in the process of calcium release³. An attempt to measure both Ca and Na gating currents in *Aplysia* neurones has been reported⁴.

We measured asymmetrical transients in neurones isolated from the snail *Helix pomatia*. The neurones were internally dialysed with potassium-free 130 mM Tris-phosphate solution +5 mM CsCl (pH 7.4). The external solution contained 120 mM Tris-HCl and 10 mM Ca and was free of Na, K and Mg. External cadmium ions were extremely effective in blocking

Fig. 1 Traces of calcium inward current (10₊, 10₋; left column) and corresponding asymmetrical transients (50₊, 50₋; right column). For the right column the Ca inward current was blocked by 2 mM Cd. The test potentials are indicated. Current calibrations were calculated for a single sweep. Holding potential -60 mV.



calcium inward current (K_d 7.2×10^{-5} M) (ref. 5). Therefore 2 mM CdCl_2 was added to the external solution to ensure complete and reversible block of the calcium current. All experiments were performed at 20–22 °C.

We used a modified version of the intracellular dialysis method described before⁶, including voltage clamp improvement, in this investigation. The main purpose of the modification was to increase the rate of the capacitive charging process. The isolated cell being fixed in the pore was impaled with a potential measuring microelectrode. This procedure minimised the error due to the series resistance. Further increase in the rate of response was achieved by a feedback technique suggested by Katz and Schwartz⁷, which gave better results than the conventional method of compensation for series resistance. Some experiments were performed without the additional feedback. The potential reached 95% of its final value in 150 μs with Katz and Schwartz compensation, in 350 μs with conventional compensation and in 1 ms with no additional feedback.

The calcium inward currents and asymmetrical transients were obtained in their net form after algebraic summation of membrane currents produced by an equal number of equal positive and negative voltage clamp pulses. Dwell time of the averaging was 50 μs . The number of pulses was usually 10 positive and 10 negative (10_+ , 10_-) for calcium currents and 50_+ , 50_- for asymmetrical transients. The interval between pulses was 1 s. In most cases positive and negative pulses were automatically alternated to ensure removal of slow inactivation. All kinds of symmetry tests were performed including averaging the pulses first from resting (-50 or -60 mV) and then from highly negative holding potential (between -100 and -150 mV). In the latter case negligible asymmetrical transients were obtained. The source of these transients has not yet been identified. They may be accounted for as the gating of fast potassium outward current, which is known to be inactivated with test pulses reaching -50 mV. The amount of charge transferred by the asymmetrical displacement current was calculated automatically by the averager which measured the area under the off-set line and then subtracted it from the area under the asymmetrical transient (for the 'on'-process) or subtracted the area under the asymmetrical transient from that under the base line (for the 'off'-process).

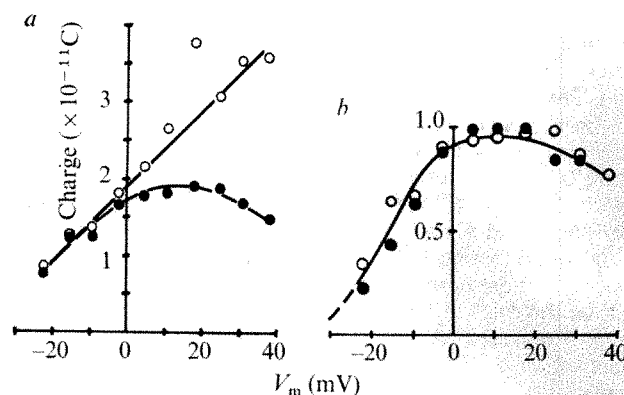


Fig. 2 *a*, Voltage dependence of charges Q_{on} (●) and Q_{off} (○) transferred by the displacement currents, calculated for a single sweep. *b*, Voltage dependence of Q_{on} (●) and calcium conductance g_{Ca} (○). Conductance was calculated for $V_{Ca} = 170$ mV (normalised). (Both *a* and *b* are from the same experiment as shown in Fig. 1).

Figure 1 shows a typical family of calcium inward current traces and corresponding asymmetrical transients obtained after cadmium block. The small inward current noticeable in the asymmetrical transient trace is due to rectification of the leakage current. The increase in test potentials above +20 mV led to the appearance of a time-dependent nonspecific outward current component. The presence of this component probably contaminated the asymmetrical transients at high test potentials by increasing the charge transferred during the 'off' pulse (Q_{off}). This is shown in Fig. 2*a* where the voltage dependence of Q_{on} and Q_{off} is presented. Q_{on} reached a peak and then tended to diminish, whereas Q_{off} persistently increased. Nevertheless, the equality of Q_{on} and Q_{off} at low test potentials allows us to identify the asymmetrical transients as displacement currents.

Figure 2*b* shows the voltage dependence of both g_{Ca} and Q_{on} . g_{Ca} was calculated as $I_{Ca}/(V - V_{Ca})$. To avoid the effect of the nonspecific outward current we obtained the reversal potential V_{Ca} from the instantaneous $I-V$ relationship at moderate test potentials. It varied between 130 and 200 mV

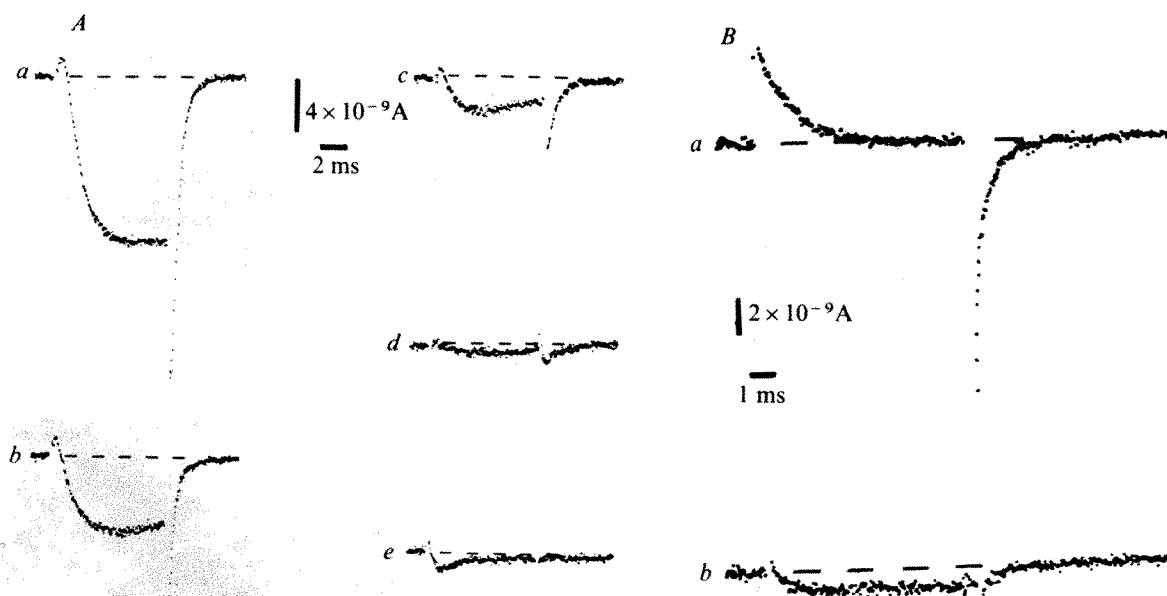


Fig. 3 *A*, The effect of fluoride on calcium inward current (*a-d*; V_t +8 mV; 10_+ , 10_-) and on the displacement current, with conventional compensation for series resistance. Averaging was performed first with Tris-phosphate inside (*a*), and then every 2 min after the application of fluoride (*b-d*). Sodium inward current (V_t -19 mV; 10_+ , 10_-) was observed after the return to normal Ringer (*e*). Holding potential -50 mV. *B*, The effect of fluoride on the displacement current. Record before (*a*) and after (*b*) adding fluoride. The feedback increasing the rate of response was introduced. Holding potential -50 mV. The negative off-set line in *b* is due to the decrease of outward going leakage current caused by internal fluoride. V_t +6 mV; 50_+ , 50_- .

for different cells. The value of V_{Ca} thus measured corresponds to that obtained for internally perfused barnacle muscle fibres⁸. g_{Ca} and Q_{on} have a similar potential dependence (Fig. 2b). This similarity, in fact, is not critically dependent on the V_{Ca} value. A decrease of Q_{on} and g_{Ca} at strong positive potential, although observed in the same potential region, could be only apparent: (1) the I_{Ca} values could be underestimated because of the nonspecific time-dependent current activation, and (2) a certain part of Q_{on} could be lost in the initial blank period because of the faster kinetics of the 'on'-process at more positive membrane potentials.

Like the calcium inward currents, the displacement currents were inactivated at more positive holding potentials. This inactivation was very pronounced, beginning with holding potential of -30 mV.

The substitution of internal phosphate by fluoride anions caused a complete and irreversible depression of asymmetrical displacement currents. It was accompanied by a decrease of outwardly directed leakage. Figure 3A demonstrates simultaneous reduction of the calcium inward current and of the initial displacement current. In this experiment the conventional compensation for series resistance was used. The delayed establishment of the final potential level led to the appearance of a rising phase in the time course of displacement current development. It is probable that the very slow rising phase of the calcium gating current described for *Aplysia* neurones⁴ is caused in a similar manner.

A small sodium inward current which was not blocked by fluoride could be detected in this cell after the return to normal Ringer (Fig. 3A). Its time course demonstrates that asymmetrical transients associated with sodium channel activation are completely lost in the 250- μ s blank period. Figure 3B shows a similar experiment but for displacement currents only and with the additional feedback.

Our data suggest that the selective block of calcium currents by internal fluoride is caused by irreversible damage to the gating mechanism of calcium channels.

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Does cyclic GMP mediate the negative inotropic effect of acetylcholine in the heart?

DURING vagal stimulation the pacemaker activity of the heart is diminished. The reduction in heart rate is due to a release of acetylcholine (ACh) from the parasympathetic nerve terminals that increases the permeability of the myocardial cell membrane for potassium ions (for review see ref. 1). This is accompanied by a shortening of the action potential duration in atrial muscle and a diminished calcium uptake², which in turn results in a negative inotropic effect. Voltage clamp experiments in mammalian atrial muscle have shown that with higher concentrations of ACh not only is the potassium current augmented but also the slow inward current of calcium is reduced³. It is

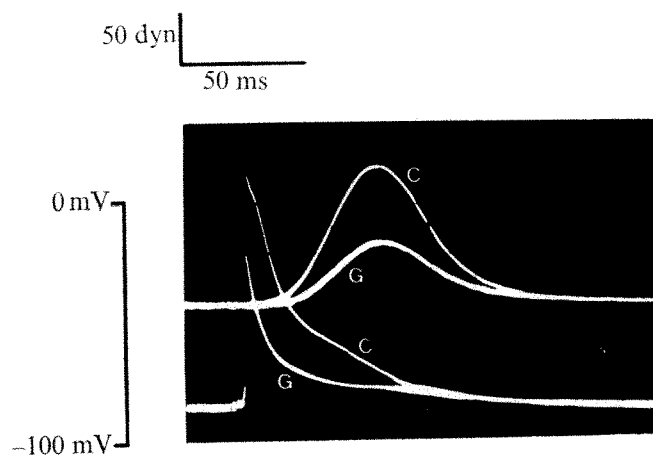
not clear how the current changes brought about by ACh are mediated. I have described the negative inotropic action of 8-bromo-cyclic GMP in the mammalian heart⁴, and these findings were confirmed in ventricular fibre fragments from mouse hearts⁵. If cyclic GMP levels in the myocardial cell are linked to the effects of ACh, as George *et al.* proposed⁶, then 8-bromo-cyclic GMP in addition to its negative inotropy should produce changes in the ion movements similar to those described for ACh. I report here the effects of 8-bromo-cyclic GMP on the action potential and the presumed underlying ion movements in rat atrial muscle.

Figure 1 shows the effects of 8-bromo-cyclic GMP on isometrically developed tension and on action potential configuration. Force of contraction was reduced to about 40% of control after 60 min; at the same time, the action potential duration was shortened at all voltage levels. Whereas the upstroke of the action potential is thought to be caused by an inflow of sodium ions⁷, the repolarisation phase is determined mainly by an outflow of potassium ions⁸. During the time course of repolarisation the potential is held at more positive potentials by an inwardly directed flow of calcium ions that has been reported for different species in ventricular and atrial preparations (for review see ref. 9). This slow inward current of calcium is believed to be of major importance for the initiation and maintenance of contraction^{10,11}.

The most direct way of testing any ion movements across cellular membranes is the use of radionuclides. Figure 2 shows that the calcium uptake in beating preparations was reduced in the presence of 8-bromo-cyclic GMP. The extent of the calcium inflow during the cardiac action potential could be diminished directly by a change in the conductance for this ion. Indirectly it could be diminished by a change in the currents of sodium and potassium that could either delay or prevent full activation of the calcium conductance by a decreased sodium inflow, or turn off and accelerate the inactivation of the calcium conductance by an increase in potassium outflow. The latter is thought to be true for low concentrations of ACh³. Either of these current changes would be detected by following the ion movements of sodium and potassium.

The uptake of radioactive sodium (²²Na, determined by liquid scintillation counting) or the net content of sodium (electrolyte contents were determined by atomic absorption spectrophotometry) were not altered in the presence of 8-bromo-cyclic GMP. Therefore the negative inotropic

Fig. 1 Effects of 10^{-4} M 8-bromo-cyclic GMP on isometric force (middle traces) and transmembrane action potential (other traces) in a rat left auricle after 60 min. The preparation was driven electrically at 0.2 Hz; other conditions were as described earlier⁴. The membrane potential was recorded intracellularly by the use of glass microelectrodes filled with 3 M KCl. C, control; G, 8-bromo-cyclic GMP.



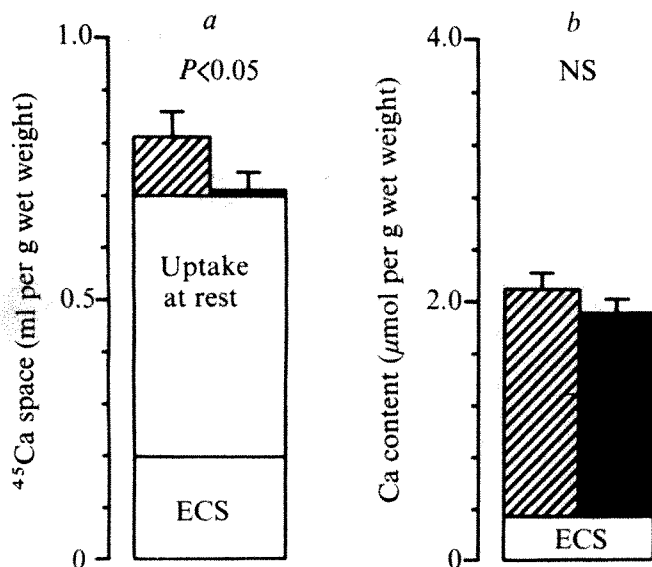


Fig. 2 Effects of 10^{-4} M 8-bromo-cyclic GMP on ^{45}Ca uptake (a) and Ca net content (b) in resting or beating (2 Hz) rat left auricles. Hatched columns, control; filled columns 8-bromo-cyclic GMP. Radioactivity was determined by liquid scintillation spectrometry after the preparations had been dissolved in 0.5 ml of tissue solubiliser, and 1 ml of 1 N HCl, plus 10 ml of scintillation fluid, had been added. The ^{45}Ca space was calculated according to Grossman and Furchgott². Each column represents the means $\pm$ s.e. of eight preparations exposed for 15 min to ^{45}Ca after 30 min of stabilisation with or without 8-bromo-cyclic GMP. All values were obtained in individual preparations. The difference in ^{45}Ca uptake was not due to a change in the extracellular space (ECS, determined by the ^{14}C -inulin method, $n = 8$); also, the uptake of ^{45}Ca while at rest was not different in controls and preparations treated with 8-bromo-cyclic GMP, $n = 16$. The measurement of the transmembrane influx of calcium related to contraction is hindered by the influence of the extracellular space and the large amount of calcium taken up while at rest. These difficulties have been described by Grossman and Furchgott². The representation of resting uptake and extracellular space should demonstrate the large influence of these 'background values' on the measurements in beating preparations. Standard errors were omitted for clarity because these values were obtained in different preparations and not considered in the statistical evaluation of results. The net content of calcium was determined by atomic absorption spectrophotometry in 16 preparations, following the same time schedule and then ashing the preparations in a 1:1 mixture of HNO_3 : HClO_4 at 180°C and redissolving the ash in N/10 HCl solution containing 1% LaCl_3 .

action of 8-bromo-cyclic GMP does not seem to be accompanied by any change in sodium movements. Indeed, a change in ion fluxes was more expected with respect to potassium, because ACh is known to induce changes in the movements of this ion. Figure 3 shows, however, that potassium efflux did not change in the presence of 8-bromo-cyclic GMP; also, the net content of potassium was not significantly affected. The efflux curve of radioactive potassium in control muscles was virtually identical to that obtained in the presence of 8-bromo-cyclic GMP and was best fitted by the equation

$$\ln Y = \ln (100 - 0.031t)$$

In resting preparations, there was also no difference in the rate of efflux of ^{42}K with or without 8-bromo-cyclic GMP. The rate constant of potassium extrusion was slightly smaller than in beating preparations and amounted to $0.026 \pm 0.001 \text{ min}^{-1}$ (mean $\pm$ s.e., $n = 8$). In identical conditions, as described in the legend to Fig. 3, ACh increased the rate of potassium extrusion by a factor of about 1.3–1.4 (Fig. 4). Because maximally effective concentrations of both 8-bromo-cyclic GMP and ACh were used in the ^{42}K washout experiments, a quantitative difference in the cyclic nucleotide and acetylcholine sensitivity is unlikely.

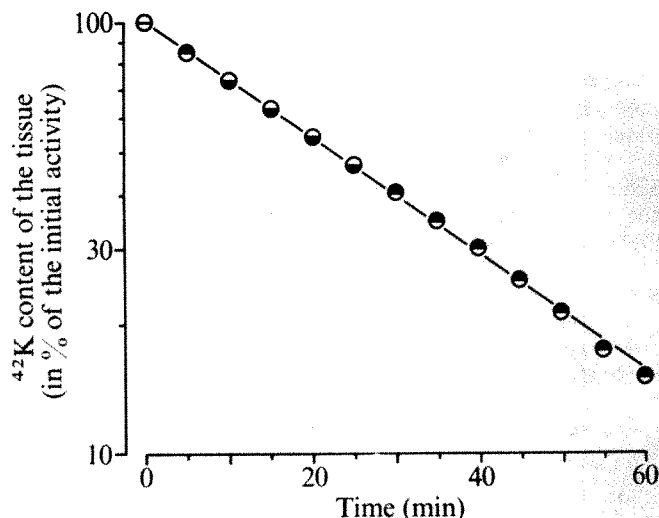
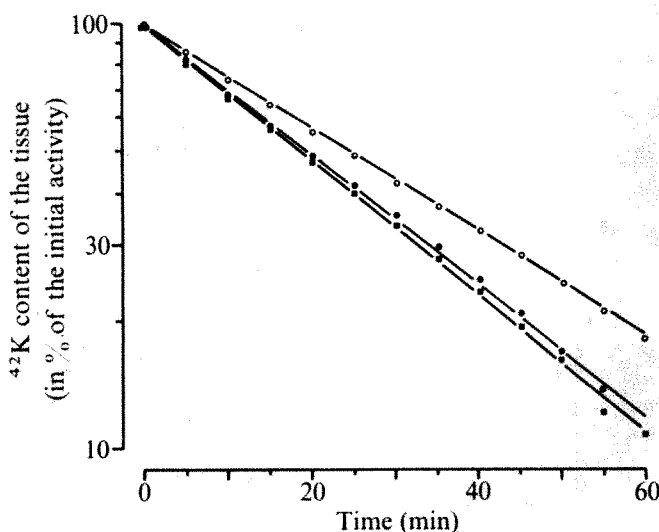


Fig. 3 Effects of 10^{-4} M 8-bromo-cyclic GMP on ^{42}K efflux in rat left auricles electrically stimulated at 2 Hz. $\circ$, Control; $\bullet$, 8-bromo-cyclic GMP. Radioactivity was determined by liquid scintillation spectrometry and by γ -scintillation counting. Symbols represent means $\pm$ s.e. of six preparations each (the values including s.e. did not vary to a greater extent than the size of the symbols used). Control and test values were obtained in individual preparations that were loaded with ^{42}K for 90 min first; then, the amount of ^{42}K released by the muscle into non-radioactive Tyrode solution was determined each 5 min; after 60 min, the experiment was finished and the residual activity in the muscle was determined. The ^{42}K content of the muscle was calculated by cumulative addition of all released amounts of radioactivity for any time the ^{42}K effluent from the preparation had been determined. The theoretical curve (the equation is given in the text) was obtained from plots of log concentration against time, using the method of least squares.

The results show that the negative inotropic effect of 8-bromo-cyclic GMP can be explained solely on the basis of an alteration in the calcium influx in beating preparations. A corresponding change is seen in the configuration of the action potential. There is no indication that the potassium system is affected by 8-bromo-cyclic GMP in the way identified for ACh. Given that in voltage clamp experiments ACh also reduced the slow inward current of

Fig. 4 Effects of ACh on ^{42}K efflux in beating rat left auricles. $\circ$, Control; $\bullet$, 10^{-6} M ACh; $\blacksquare$, 5×10^{-6} M ACh. Experimental procedure was as described in Fig. 3. Means $\pm$ s.e. of rate constants of the washout process amounted to 0.028 ± 0.002 (control, $n = 4$), 0.036 ± 0.003 (10^{-6} M ACh, $n = 3$) and 0.038 ± 0.003 (5×10^{-6} M ACh, $n = 4$) min^{-1} . Correlation coefficients for all experiments varied between 0.996 and 0.999. Significance level for the difference between the washout curves obtained in control conditions and ACh was $P < 0.05$.



calcium in mammalian³ and frog^{12,13} atrial muscle, it is possible that the effects of ACh are mediated in part by changes in the intracellular levels of cyclic GMP; on the other hand, another pathway by which ACh mediates its physiological effects seems to be obvious.

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Variation of thin filament length in heart muscle

ALTHOUGH the length dependence of force generation by heart muscle has been attributed to the degree of overlap of thin and thick filaments within the sarcomere, the required accurate values of the lengths of cardiac thin filaments have not been available. The existence in frog atrial trabeculae of thick filaments of uniform 1.55 μm length, of a small number of very long thin filaments, and of rosettes containing fewer or more than six thin filaments surrounding each thick filament has been noted¹ (Fig. 1), but no study of the dimensions of a statistically significant number of thin filaments has been made. Using serial transverse electron micrographs of cardiac cells we have found that the lengths of thin filaments vary by as much as 0.6 μm within a single sarcomere in a single myofibril. All other factors being constant, this broad range should increase the length of the plateau of the sarcomere length-tension relationship and allow the myocardial cells contracting *in situ* to operate entirely on the plateau. The sliding of

Fig. 1 Electron micrograph of a transverse section of rat atrial muscle. A small number of long thin filaments extends into the hexagonal M bridge and thick filament array. Thick filaments are surrounded by 0–6 thin filaments. Total magnification: $\times 121,000$.

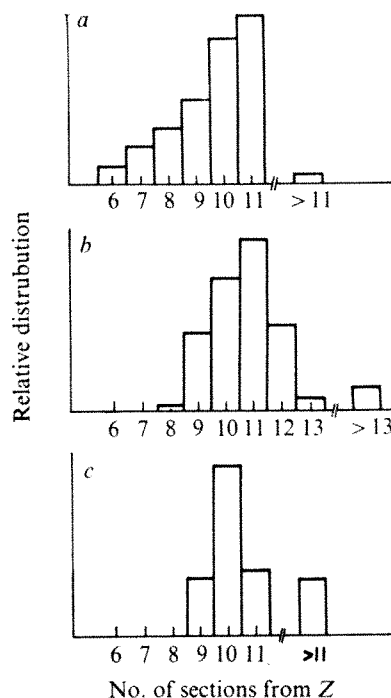
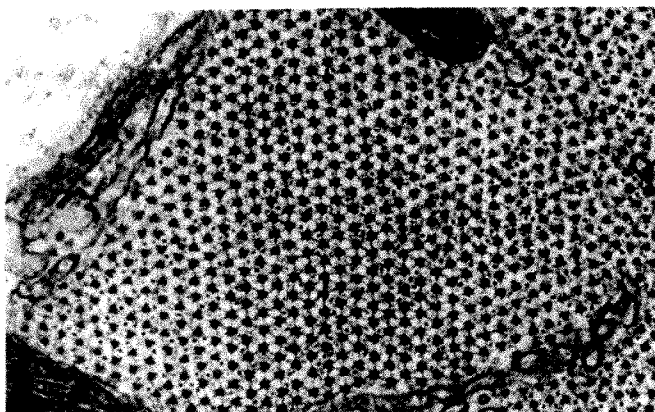


Fig. 2 Relative frequency of thin filament lengths which are expressed in number of sections from the flat Z band for (a) rat atrial trabeculae, (b) frog atrial trabeculae, and (c) rat papillary muscle. The estimate of the section thicknesses was 0.1 μm whether it was based on the pale gold interference colour of the freshly cut, floating sections or derived from the known 1.5 μm length of the thick filament, which was used as an internal calibration standard that compensated for any generalised filament shrinkage⁶. The width of each histogram bar represents the section thickness and thus the limit of resolution of the technique.

myofilaments, therefore, should have little direct effect on force generation in the intact heart.

We have measured the lengths of populations of thin filaments in frog and rat atrial trabeculae and rat papillary muscle by analysing electron micrographs of serial transverse sections through a major portion of sarcomeres of muscles which were stretched and held throughout the preparative procedures of fixation and dehydration. Two different regions from each tissue were studied. Longitudinal sections are unsuitable for estimating filament length because in thick sections there is no discernible H zone, and in very thin sections there are the dangers of producing errors by either clipping off part of the filaments or superimposing filaments. To eliminate artifacts associated with the use of the approximately 0.1 μm thick transverse serial sections, regions were used only when the Z band was flat and entirely included within one section; when all filaments were absolutely perpendicular to the plane of section without using any tilting of the specimen stage; and when at least 400 thin and 200 thick filaments could be followed in a given region from one section to another. The numbers of thin and thick filaments were then counted in each transverse section and the relative lengths of thin filaments from the Z band deduced from the ratios of thin to thick filaments. Estimates of absolute values for filament lengths were determined by multiplying the number of serial sections through which the filaments extended by 0.1 μm , the approximate thickness of a section. Similar values were produced by using the length of the thick filament as an internal calibration. Values for the ratios of the number of thin to the number of thick filaments varied between 0 and 2. The maximum value of 2 attests to the suitability of the sarcomere for filament length analysis; a value greater than 2 would indicate poor lateral alignment of thick filaments, whereas a value less than 2 would show that some of the shortest thin filaments did not reach the A band

lattice as a result of overstretching of the muscle. Since the absolute number of thick filaments is constant throughout the A band, the relative numbers of thin filaments of each length have been obtained from a comparison of the ratios of thin to thick filament densities in each section. Typical data are shown in Fig. 2.

Thin filament lengths in frog atrial trabeculae varied from eight to more than thirteen section thicknesses, a span of more than $0.6\text{ }\mu\text{m}$, and in rat atrial trabeculae the range was equal to over six section thicknesses, or more than $0.6\text{ }\mu\text{m}$. In the rat papillary muscle, however, thin filaments differed in length from nine to more than eleven section thicknesses, or about half the spread of atrial tissue. The wide range of thin filament lengths found within a single sarcomere of a single myofibril indicates that there is no unique value for the extent of overlap even within one sarcomere. Consequently, any relation between length and force generation will be dependent not only on sarcomere length but also on the distribution of lengths of thin filaments.

An assessment of the effects of the broad distribution of lengths of thin filaments on the relationship between sarcomere length and developed force can be made if the data are coupled with the observations of Gordon *et al.*⁵, relating force to certain relative positions of thick and thin filaments. The calculations involved construction of sarcomere length-tension curves for each thin filament length, weighting by the appropriate factor from the length distribution, and summation of all the weighted curves to give a composite, calculated sarcomere length-developed tension curve. The exact basis for the corner between the ascending limb and the plateau has not been well defined;

the decrease in developed tension with sarcomere shortening past the plateau might be due to such things as interference of cross-bridge activity in the vicinity of a larger than usual number of actin filaments or the appearance of an internal resistance from the mutual interaction of thin filaments from opposite sides of the sarcomere during double overlap. Separate calculations based on decreased generation of force from either the sliding of a thin filament past the bare zone and into the opposite half of the A band or the double overlap of thin filaments from opposite sides of the sarcomere produced only slightly different results.

Figure 3a and b show the relations that were calculated using the first and simpler of the two modelling possibilities. The composite sarcomere length-developed tension curves of the atria have shapes distinctly different from that of skeletal muscle, particularly with respect to their much broader plateau regions. The wide range of thin filament lengths in atrial muscle provides a nearly constant degree of functionally effective thin and thick myofilament overlap over the physiological working range, and the sliding of filaments with changes in sarcomere length should therefore have little direct effect on the generation of tension.

The presence of a broad zone with only a very shallow slope in the length-tension relationship of calcium-activated, mechanically skinned cardiac fibres⁷ is consistent with the observed dimensions of thin filaments, and the absence of a plateau for the same relation in isolated cardiac muscles^{8,9} indicates that the modulation of force in the intact tissue must result from another step in the contractile cycle, such as activation⁷.

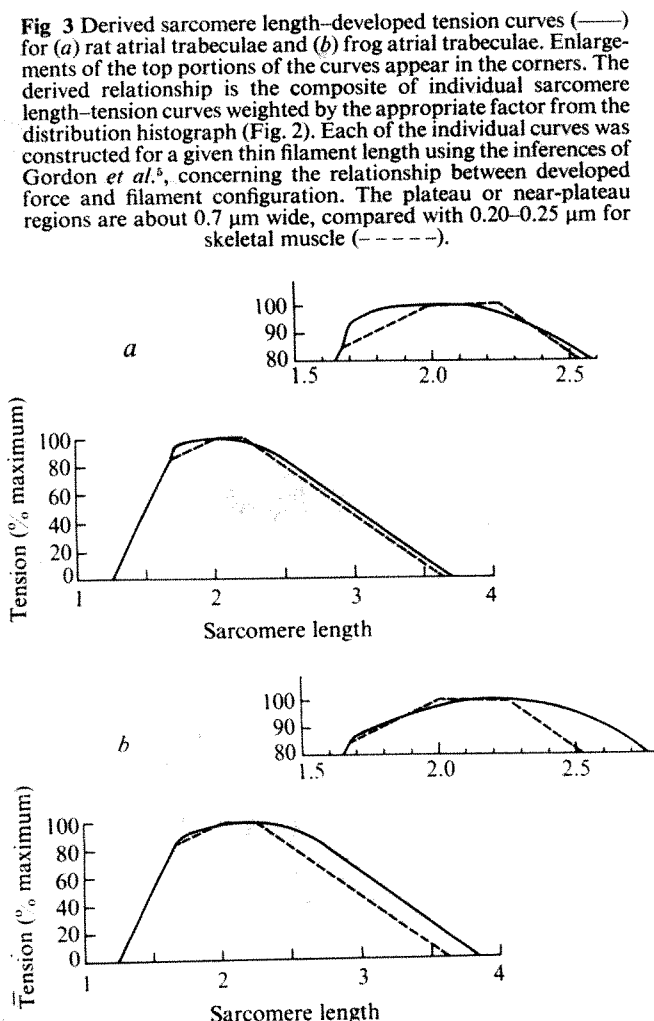
Of additional interest in this study was the correlation between the compliance of the tissue and the breadth of the distribution of thin filament lengths (Fig. 2); the stiffer papillary muscle had the narrower distribution. This raises the possibility that the breadth of the distribution may actually be set by the cell according to the amount of shortening which the cell undergoes in the intact heart to produce a plateau of adequate length in the sarcomere length-tension curve.

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Sodium sensitivity of baroreceptors mediates reflex changes of blood pressure and urine flow

CARDIOVASCULAR baroreceptors are sensitive to vascular pressures and their input to the central nervous system is essential for the regulation of arterial blood pressure. They also contribute to the regulation of extracellular fluid volume, because their reflex effects upon arterial pressure and renal blood flow have marked consequences for urine flow^{1,2}. Since they are mechanoreceptors their properties may be affected by sodium and potassium ions³⁻⁵. Lowering extracellular Na^+ (Na_o^+) increases the threshold pressure at which arterial baroreceptors begin to discharge and reduces their sensitivity to suprathreshold pressures^{6,7}. We therefore investigated the reflex effects upon arterial blood pressure

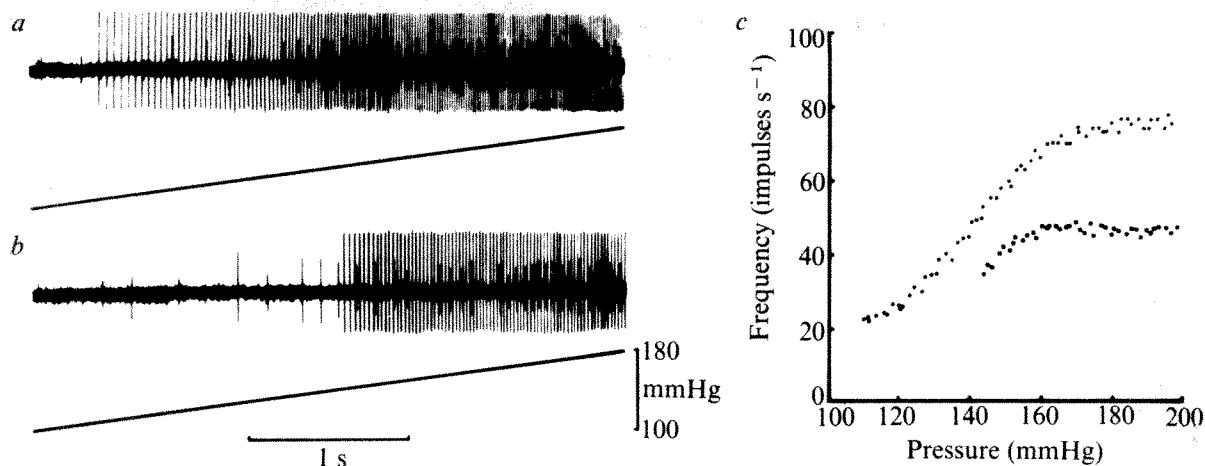


Fig. 1 Effects of lowering extracellular Na^+ (Na_o^+) on baroreceptor discharge. *a*, The tall spikes in the top trace are the neural discharge of a single baroreceptor in control solution and the slowly rising line in the bottom trace is a pressure ramp of 20 mmHg s^{-1} . *b*, Same arrangement as (*a*) but the baroreceptor is now in $50\% \text{ Na}_o^+$ solution. The threshold pressure is clearly increased and maximum discharge frequency is reduced. *c*, Discharge frequency–pressure plot of the neural discharge shown in *a* and *b*. Each point on the frequency against pressure plot is the inverse period between two successive spikes. Filled circles (left hand curve) and filled rectangles (right hand curve) represent the neural discharge patterns shown in (*a*) and (*b*), respectively.

and urine flow elicited by lowering the Na_o^+ of carotid sinus baroreceptors. We report that reductions in Na_o^+ of as little as 5% increase arterial blood pressure and urine flow. We propose that the sodium sensitivity of baroreceptors is important for their function as regulators of arterial blood pressure and body fluid volume.

Figure 1*a, b* shows the pressure–discharge curves of a single rat aortic arch baroreceptor in control and $50\% \text{ Na}_o^+$ solution. Composition of the control solution was, in mM: Na^+ , 145; K^+ , 6; Cl^- , 127; Ca^{2+} , 1.1; Mg^{2+} , 1.2; HCO_3^- , 25; H_2PO_4^- , 1.2; SO_4^{2-} , 1.2; dextrose, 5.5. Na_o^+ was usually replaced by Tris [Tris (hydromethyl) aminomethane] at constant osmolality in the 95% and 87.5% Na_o^+ solutions and at a measured increase of less than 1% in the 75% Na_o^+ solutions and 2% or less in the 50% Na_o^+ solutions. Neural discharge was elicited by applying pressure ramps of $4\text{--}20 \text{ mmHg s}^{-1}$ to the aortic arch. Slow ramp rates such as these generate pressure–discharge curves which are very similar to those elicited by steady state pressure steps. Figure 1*c* shows the ‘instantaneous’ frequency versus pressure plots for the discharges and pressures shown in (*a*) and (*b*). Lowering Na_o^+ clearly increases the pressure for threshold discharge

and reduces the sensitivity to suprathreshold pressures. Seventeen individual baroreceptors studied in this manner showed similar responses to reductions in Na_o^+ of 25–50%.

The steady state pressure–volume relationship of the aortic segments used in these experiments was measured as described previously^{8,9}. The pressure–volume curves were unchanged by the test solutions so that the ionic actions were directly upon the receptors. In addition, the rate of rise of pressure in the isolated aortic segments elicited by fixed input ramp rates was unchanged in the test solutions.

The reflex experiments were performed on cats which had been in the vivarium for at least 2 d. They were fed Purina cat food and allowed to drink water freely. The animals were anaesthetised with a mixture of chloralose (Baker) 50 mg per kg body weight, and urethane (Matheson, Coleman & Bell), 200 mg per kg, injected intraperitoneally. The cervical vagi were cut bilaterally and one carotid sinus nerve was cut. The other carotid sinus was isolated and perfused via polyethylene tubing coupled to a Holter pump which was connected to a reservoir containing Krebs–Henseleit solution bubbled with 95% O_2 and 5% CO_2 and heated to 37°C . The perfusate was collected from the external carotid artery so that none

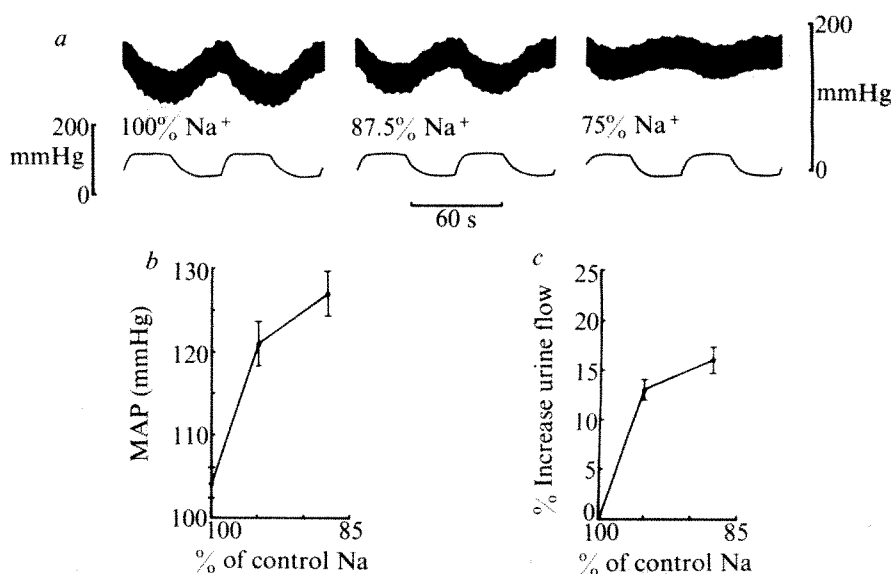


Fig. 2 Reflex effects of lowering Na_o^+ in the carotid sinus perfusate on arterial blood pressure and urine flow. *a*, Top trace is systemic arterial blood (mean arterial) pressure (MAP) and bottom trace is left carotid sinus pressure. Repetitive step increases in sinus pressure elicit repetitive falls in arterial pressure. As Na_o^+ in the carotid sinus is reduced at constant intrasinus pressure steps, mean arterial pressure rises and the oscillations in pressure elicited by constant steps are reduced. *b*, Mean arterial blood pressure as a function of carotid sinus Na_o^+ at constant mean carotid sinus pressure. Data are from nine animals. *c*, Plot of the % increase in urine volume over control as Na_o^+ is varied. The control urine flows were $39 \pm 0.003 \mu\text{l min}^{-1}$.

of it was added to the cat's circulating blood volume. Overdamped pressure steps could be superimposed by a parallel connection with a shaker pot (Ling 411) (Fig. 2a). Pressure in the perfusion circuit was measured using a Statham P23dB strain gauge manometer. Arterial blood pressure was measured by inserting a polyethylene catheter into a femoral artery. The catheter was connected to a Statham P23dB strain gauge manometer. The animal was given an intravenous injection of 0.9% NaCl equal in volume to 1.0% of body weight over 20 min at the beginning of the dissection. This was followed by continuous infusion of Krebs-Henseleit at 5 ml h^{-1} . Urine output was measured in timed fractions of 8–16 min duration by collecting the urine flowing from polyethylene catheters which were inserted into the ureters. Urine sodium concentration was measured using sodium glass miniature electrodes, or by an atomic absorption spectrophotometer (Instrumentation Laboratory IL253). The sodium glass was Corning NAS11-18, had a Nernstian response for Na^+ and a selectivity of 50:1 and 100:1 respectively over K^+ and NH_4^+ .

The isolated carotid sinus was perfused with the control solution until mean arterial blood pressure and urine flow became steady. This period was about 30 min. When the Na_o^+ in the sinus perfusate was lowered to 95% or 87.5% at constant mean sinus pressure of 90–100 mmHg, mean arterial blood pressure always increased (Fig. 2b) and urine flow was always enhanced (Fig. 2c). The changes in arterial blood pressure elicited by applying overdamped pressure steps to the isolated carotid sinus were also reduced in amplitude (Fig. 2a). The relationship between the Na_o^+ concentrations in the sinus perfusate and the changes from control of mean arterial blood pressure, and urine flow measured in nine animals, are shown in Fig. 2b and c. The increases in arterial pressure and urine flow evoked by the various test Na^+ solutions were statistically significant at the 0.01 level. Recovery of blood pressure and urine flow to control values occurred between each test solution. The test solutions did not change the distensibility of the common carotid artery or the isolated carotid sinus and we conclude, as we did for rat aortic baroreceptors, that the ionic effects are due to a direct action upon the baroreceptors.

The role of the renal sympathetic nerves in the diuresis was examined in another six animals. One kidney in each animal was denervated and urine flow and sodium excretion were compared between the innervated and denervated kidneys of the same animal. Denervation did not affect control arterial blood pressure; a small but not significant increase in urine flow did occur¹⁰ (Fig. 3). Na^+ excretion which varied considerably was reduced but the change was not significant (Fig. 3). Lowering Na_o^+ to 87.5% in the isolated sinus always increased arterial blood pressure and urine flow and the increases were statistically significant. The diuresis was not different, however, between the innervated and denervated kidneys. Sodium excretion was not altered significantly by the test sinus solutions (Fig. 3).

The action of reduced Na_o^+ upon the carotid sinus baroreceptors is not due to an osmotic effect since the osmotic pressure was unchanged by Tris substitution for Na_o^+ at the 5% and 12½% levels and only slightly increased at 25% and 50% levels. Nor is the effect likely to be related to an action of Tris since replacement of 5% or 12½% NaCl with isosmotic sucrose (four experiments) gave the same response as substituting Tris for Na^+ . It is possible that lowering Na_o^+ inhibited carotid body afferent discharge. The reflex effects on blood pressure would tend to offset the reflex effects of reducing baroreceptor input so that the sodium sensitivity of the baroreceptor reflexes might be even greater than the present experiments

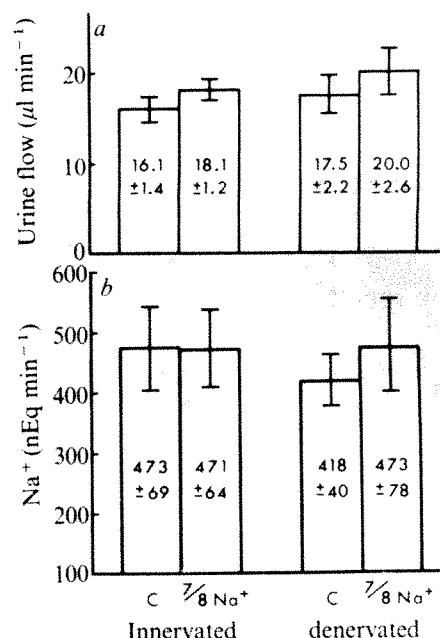


Fig. 3 Comparison between innervated and denervated kidneys of the effects of lowering carotid sinus Na_o^+ . *a*, The urine volumes are compared for innervated and denervated kidneys from six animals when the isolated carotid sinus was perfused with control solution, C, and when it was perfused with 7/8 Na_o^+ . There was a significant difference ($P < 0.01$) between the effects of control solution and 7/8 Na^+ upon urine flow in both kidneys but there was no significant difference between innervated and denervated kidneys. *b*, The total urinary sodium output when the sinus is perfused with control and 7/8 Na^+ . Six animals were compared in this way. There were no significant differences between the effects of the two solutions on innervated or denervated kidneys.

indicate.

The sensitivity to Na_o^+ was greater in the reflex experiments than in the individual baroreceptor discharge experiments⁷. This is undoubtedly due to the fact that in the reflex experiments all of the baroreceptors are tested simultaneously. The experiments using individual baroreceptors are complicated by the variability in threshold and sensitivity among different receptors as well as a 1–2% variability in individual receptors.

Arterial baroreceptors respond to small decreases in extracellular Na^+ concentration by producing a reflex increase in arterial blood pressure and urine flow. Sodium excretion seems to be unchanged but, because of its variability, this point is not definitely established. The mechanism of the diuresis is unclear. The renal nerves do not seem to be essential and changes in antidiuretic hormone (ADH) secretion are unlikely to be involved since ADH is increased by reductions in baroreceptor discharge¹¹. The increase in arterial blood pressure is a likely possibility¹². Sodium sensitivity of baroreceptors may be an important mechanism for prompt regulation of body fluid volume, and ionic sensitivity of cardiovascular baroreceptors is possibly as important to body function as their pressure sensitivity.

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Interaction of DNA and liposomes as a model for membrane-mediated DNA damage

We have described the antioxidant activity of calf thymus DNA on the oxidation of liposomal suspensions¹; we now report the damaging consequences to the biological activity of bacterial transforming DNA in such a system. Because DNA is associated with eukaryotic² and bacterial cell membranes³, the DNA-membrane interaction described here may be relevant to several pathological processes, including carcinogenesis⁴⁻⁷, ageing⁸⁻¹¹ and oxygen-dependent, radiation-induced cell lethality^{10,11}. In the presence of oxygen, the unsaturated fatty chains of membrane phospholipid undergo autoxidation¹². These autocatalytic free radical reactions, due to their chain nature, both amplify and propagate an initiating event occurring within the membrane, during which time many highly reactive and potentially damaging chemical species are formed¹³.

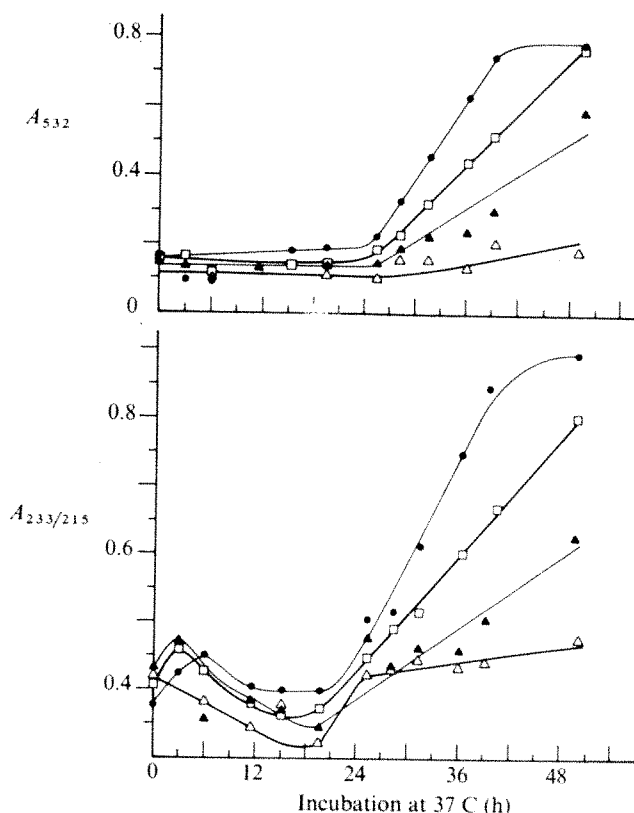
In view of the previous findings, we decided to investigate the possibility that retardation by DNA of the oxidative free radical reactions occurring within membrane phospholipids¹ would damage the DNA. Incubation mixtures of liposomes and *Bacillus subtilis* or calf thymus DNA were studied. Because liposomes are highly organised bilayers, they provide a model of *in vivo* membrane phospholipid configuration¹. The content of polyunsaturated fats was increased by enrichment with methyl arachidonate to enhance the rate of lipid oxidation in the bilayer system¹. Lipid oxidation was monitored colorimetrically by the thiobarbituric acid method (A_{532}) and spectroscopically by diene conjugation ($A_{233/215}$). DNA damage was assayed by measuring the reduction in transforming activity of *B. subtilis* BD 170 (thr 5 trp 2) cells. Figure 1 shows the effect of various concentrations of calf thymus DNA on the oxidation kinetics of methyl arachidonate-enriched phosphatidyl choline liposomes (PCMA)¹. None of the DNA concentrations studied increased the induction period, which in this experiment was approximately 21 h. The DNA did, however, retard the rate of lipid oxidation in a concentration-dependent manner. This and other results (ref. 14 and personal communication from K. M. Schaich and D. C. Borg) suggest that DNA interacts with the oxidising model membrane system as a free radical acceptor or trapping agent^{1,13}. Figure 2 shows the lipid oxidation kinetics of phosphatidylcholine (PC) and PCMA liposomal suspensions containing *B. subtilis* transforming DNA ($4.5 \mu\text{g ml}^{-1}$). The fatty chain profile, buoyant density characteristics and oxidation kinetics of these liposomes have been described before¹. The induction period for each suspension is the same, although the PCMA liposomes oxidise at a greater rate because of their higher degree of polyunsaturation. At the times indicated by the arrows in Fig. 2, DNA was isolated from each suspension and assayed for transforming activity (Table 1). DNA incubated in either sodium phosphate buffer or standard saline citrate (SSC) was stable during 68 h incubation, but DNA incubated with the oxidising liposomal suspensions lost its transforming activity in a manner dependent on the magnitude of lipid oxidation which has occurred in each suspension up to the time of sampling. In this respect, PCMA liposomes decreased the transforming activity of DNA by a factor of 2 during 16 h incubation, while the more slowly oxidising PC liposomes

caused no DNA damage. By 36 h incubation, PCMA liposomes had caused DNA transforming activity to decrease by a factor of 3,000 (the limit of the system), while PC liposomes had caused it to decrease by a factor of 8, again reflecting their lower oxidation rate relative to PCMA liposomes. After incubation with DNA for 68 h, PC liposomes also caused the largest detectable decrease in DNA transforming activity, by a factor of 3,000.

We also incubated *B. subtilis* DNA with PC and PCMA liposomes in SSC. During 68 h incubation, neither PC or PCMA underwent autoxidation, and accordingly DNA incubated in these non-oxidising systems retained its full transforming activity throughout the 68 h. Furthermore, fully saturated dipalmitoyl phosphatidyl choline liposomes suspended in sodium phosphate buffer underwent no autoxidation during 68 h incubation at 45 °C, and, correspondingly, *B. subtilis* DNA retained full transforming activity when incubated with these suspensions during that time. These controls show that non-oxidising membrane lipid *per se* has no effect on the transforming activity of DNA; for damage to occur, the lipids must be oxidising.

Taken together, these results indicate that oxidation of highly organised lipid bilayers can damage DNA. The species of oxidising lipid responsible for this damage are unknown but could be free radicals or reactive non-radical compounds. Free radicals, such as the $\cdot\text{OH}$ radical, which are produced during lipid autoxidation, have been shown to damage DNA¹⁵. The finding that DNA acts as a radical trapper in the model systems described before would support the hypothesis that DNA damage is caused, at least

Fig. 1 Oxidation kinetics of PCMA liposomal suspensions containing various concentrations of calf thymus DNA. PCMA liposomes were incubated alone or with various concentrations of dialysed calf thymus DNA in 0.05 M sodium phosphate buffer pH 7.5, as previously described¹. Total lipid concentration was 10 mM. Lipid oxidation was monitored by both A_{532} and $A_{233/215}$. Each point plotted represents the average of readings taken from two identically prepared liposomal incubation samples during a typical experiment. These results have been repeated three times. ●, PCMA; □, PCMA+DNA ($4.5 \mu\text{g ml}^{-1}$); ▲, PCMA+DNA ($45 \mu\text{g ml}^{-1}$); △, PCMA+DNA 225 ($\mu\text{g ml}^{-1}$).



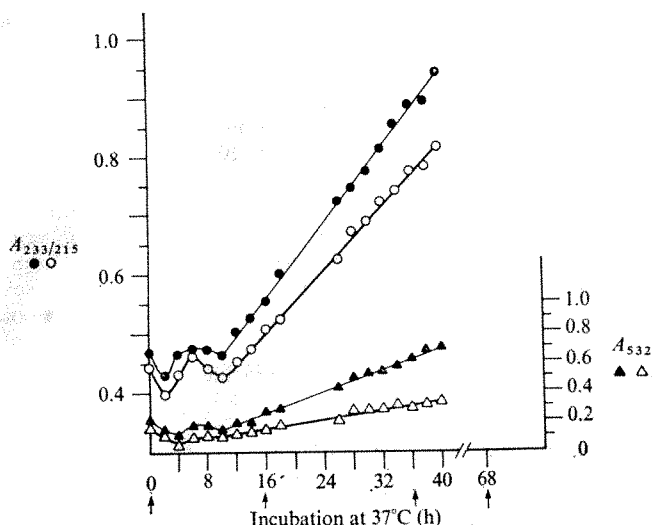


Fig. 2 Oxidation kinetics of PCMA and PC liposomal suspensions each containing *B. subtilis* DNA. PCMA and PC liposomes were incubated with *B. subtilis* ^3H -DNA ($4.5 \mu\text{g ml}^{-1}$) in 0.05 M sodium phosphate buffer pH 7.5, as described for PCMA liposomes and calf thymus DNA¹. Transforming ^3H -DNA (specific activity 2,000 d.p.m. μg^{-1}) was isolated from *B. subtilis* BD 204 (his B2 thy) (given by D. Dubnau) grown for four generations in SPI medium²⁹ containing L-histidine hydrochloride hydrate ($50 \mu\text{g ml}^{-1}$), thymidine ($1.5 \mu\text{g ml}^{-1}$) and $1.75 \mu\text{Ci } ^3\text{H}$ -thymidine ml^{-1} (19 Ci mmol^{-1}) by the Sepharose 4B procedure³⁰. Total lipid concentration was 10 mM in each suspension. Both A_{532} and $A_{233/215}$ were assayed to monitor lipid oxidation. Each point plotted is the average for four independent samples. The arrows indicate the times when samples were assayed for DNA transforming ability. ●, ▲, PCMA+DNA; ○, △, PC+DNA.

in part, by this radical attack. The carbonyl malondialdehyde is a typical non-radical, reactive compound formed during lipid peroxidation¹³. Malondialdehyde binds to DNA, decreasing its ability to serve as a template for RNA polymerase¹⁶. While unorganised emulsions of oxidising polyunsaturated fatty chains have been shown to damage DNA in conditions that enhance such carbonyl-DNA interaction (pH 4.6)¹⁷, the defined structural ordering in the membrane models used in the study reported here as well as the difference in pH between it and the previous work¹⁷, would make extrapolation difficult.

It has been suggested that membranes are the site of oxygen enhancement of radiation-induced cell lethality (the so-called "oxygen effect")^{10,11}. Supporting evidence includes

Table 1 Transforming activity of *B. subtilis* DNA after incubation with PC or PCMA liposomes

Incubation time (h)	DNA	Sample incubated DNA+PC	DNA+PCMA
0	3,250	3,320	2,850
16	2,690	3,105	1,471
36	3,190	427	0
68	3,030	0	0

At the times indicated by the arrows in Fig. 2 and listed above, a 0.5-ml sample was removed from each incubation sample, added to 10 ml cold absolute ethanol and centrifuged at 15,000 r.p.m. for 20 min at 4 °C. The resulting BD204 ^3H -DNA pellet was resuspended in 0.5 ml sodium phosphate buffer, pH 7.5. After 0.1 ml was counted to determine the ^3H -DNA concentrations, a volume corresponding to $0.45 \mu\text{g}$ of ^3H -DNA (0.15 ml) was assayed for ability to transform BD170 (thr 5 trp 2) to threonine independence²⁷. Transforming activity is the number of BD170 cells transformed by $0.45 \mu\text{g}$ of BD204 ^3H -DNA per 5×10^7 exposed cells. Incubation samples consisted of BD204 ^3H -DNA ($4.5 \mu\text{g ml}^{-1}$) alone in either 0.05 M sodium phosphate buffer, pH 7.5, or 0.015 M NaCl and 0.0015 M sodium citrate (SSC), pH 7.0, (DNA); or mixed suspensions of BD204 ^3H -DNA ($4.5 \mu\text{g ml}^{-1}$) with 10 mM PCMA (DNA+PCMA) or 10 mM PC (DNA+PC) in 0.05 M sodium phosphate buffer, pH 7.5, as shown in Fig. 2. Each value is the average for four independent samples. The standard deviation for each value is $\leq 10\%$.

the effects of lipid-soluble inert gases¹⁸, the "time resolved O_2 effect"¹⁹, and the large O_2 effect on DNA-membrane complexes²⁰. In addition, ionising radiation initiates the oxidation of ordered micellar fatty chains with a G value of approximately 100 (ref. 21). Hence the type of membrane-mediated DNA damage described here is a possible mechanism of the O_2 effect, and the markedly higher solubility of oxygen in lipid, compared with water, makes this suggestion even more attractive²².

The relevance of this type of interaction in carcinogenesis is supported by the findings that both lipid peroxides and malondialdehyde are mutagenic^{23,24} and carcinogenic^{23,25}. Therefore, the components necessary to produce carcinogenic insult, that is O_2 and unsaturated fatty chains, are present in all cells in normal conditions. It has been proposed that lipid autooxidation is a continual process in living aerobic cells, and is restrained from entering the autocatalytic phase by enzymes and antioxidants⁸. Chemical and physical agents which enhance membrane free radical reactions may accelerate this process beyond the capabilities of those restraining systems and result in widespread membrane lipid oxidation and cell death^{13,26}.

Finally, it has been hypothesised that one cause of cellular ageing is membrane lipid oxidation-mediated damage to DNA and other macromolecules⁸. The studies described here demonstrate that oxidising membrane lipids impair the biological function of DNA and support this view.

The importance of the interaction of oxidising membrane lipids with DNA and/or other critical biomolecules in various pathological conditions can now be studied more directly using the unsaturated fatty acid-requiring auxotrophs isolated from *E. coli*²⁷ and Chinese hamster ovary²⁸ cell lines.

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Gap filling during postreplication repair of DNA in recombination deficient *Escherichia coli*

POSTREPLICATION repair of DNA is one pathway for repair of damage inflicted on DNA by physical and chemical agents in the environment¹. Postreplication repair can involve error-prone repair of pyrimidine dimers in *Escherichia coli*², and as such may be particularly relevant to the understanding of the alteration of initial damage in DNA to a transformed or mutant DNA. The assignment of gene products to essential roles for completion of postreplication repair gap filling after ultraviolet light (UV) damage in *E. coli* cells³⁻⁷ has been complemented by consideration of their respective roles in error-prone repair of UV damage⁸⁻¹¹. We have used column chromatography, which separates double-stranded DNA containing single regions from completely double-stranded DNA, to observe the gap filling during postreplication repair of UV damage to DNA. In addition, analysis of *E. coli* *recA* or *lex* mutants demonstrated that the *recA*⁺ or the *lex*⁺ gene products must control steps exclusive of the initial gap filling process during postreplication repair.

DNA synthesised immediately after exposure to UV is discontinuous¹². Closure of these daughter strand gaps can be demonstrated by analysis of alkaline sucrose density gradient profiles. DNA increases in size (filling and joining) with reincubation. Alkaline sucrose centrifugation analysis of DNA size, however, does not differentiate the initial steps in gap filling from partially completed gap filling. In contrast, benzoylated naphthoylated DEAE-cellulose (BN-cellulose) chromatography is sensitive to the single-stranded regions in the double-stranded DNA structure and thus is suitable for analysis of the progress of gap filling. BN-cellulose chromatography was used successfully by Iyer and Rupp¹³ to measure the average gap size generated by DNA synthesis on UV-damaged parental DNA. We have used the same procedure.

E. coli cells were grown, exposed to UV and labelled with ³H-thymidine, and DNA was released and sheared as described by Iyer and Rupp¹³. Caffeine elutions from the BN-cellulose column of DNA from AB3063 cells with and without UV exposure were duplications of the procedure of Iyer and Rupp and gave the same results for the same strain. The work described here had an additional step, whereby some cells were reincubated in non-radioactive medium before caffeine elution. In addition, strains deficient in *recA*⁺ gene product were studied. The same procedure was used for a strain deficient in *lex*⁺ gene product and for its progenitor. The results shown here represent means of three to seven experiments with standard errors of the mean given for the caffeine elution.

In *E. coli*, the *recA*⁺ gene product controls postreplication repair. DNA from *recA* mutant cells that have been reincubated for 60 min after UV treatment does not increase to the size of DNA from unirradiated cells³. DNA from *E. coli* *lex* mutants demonstrates a slow increase in DNA size in the same conditions⁴. The results for postreplication repair observed indirectly by BN-cellulose chromatography in this study agreed with those of alkaline sucrose centrifugation studies: a protocol producing postreplication repair of daughter strand gaps in *recA*⁺*lex*⁺ cells as indicated by gradient profile analysis resulted in a commensurate decrease in the number of daughter strand gaps indicated by BN-cellulose chromatography (Table 1). Analysis of BN-cellulose chromatography elution, however, demonstrated that *recA* or *lex* mutant strains, which are slow or unable to complete postreplication repair, were able to convert DNA with single-stranded regions to double-stranded DNA (Table 1). These experiments were repeated with additional *recA* strains including a *recA56recB21recC22* strain and isogenic *recA* and *recA*⁺ strains obtained from Dr S. D. Barbour (not shown), and the results indicated that wild type cells and mutants could convert equal amounts of gapped DNA to double-stranded DNA. Thus, while *recA*⁺ and *lex*⁺ gene products are essential for normal levels of postreplication repair, mutants demonstrate extensive conversion of UV-induced postreplication daughter strand gaps to double-stranded DNA.

Howard-Flanders¹ and Hanawalt¹⁴ have reviewed the possible mechanisms of postreplication gap filling. They discussed the roles, relative to the gap filling process, of *de novo* DNA synthesis, DNA strand exchange and the process by which gap filling is initiated. Our results support the hypothesis that the *lex*⁺ and *recA*⁺ gene products in wild type cells are essential for postreplication repair gap closure after initiation of gap filling and/or the first steps thereof. Essentially, the function of the error-prone repair *recA*⁺ or *lex*⁺ gene product seems to be the filling of the minority of the gap.

An alternate explanation for the gap filling observed in *E. coli* *recA* or *lex* mutants could involve preferential degradation of the single-stranded parental DNA forming the gap backbone. Although the stability of the parental DNA was not measured in the experiments shown here, degradation might be below detectable levels even if the parental DNA were labelled. Nevertheless, such degradation would precipitously decrease the size of parental DNA as measured with alkaline sucrose gradient analysis during post-UV reincubation of *recA* mutants. This has not been observed in gradient analysis in this laboratory. More importantly, in the *recArecBrecC* mutants, with greatly reduced degradation ability, loss of daughter strand gaps was observed as with the *recA* mutant (not shown).

An alternative hypothesis is that gap filling represents the function of proteins produced by leaky mutants. If, however, the *recA*⁺ gene product were responsible for the control of all the steps

Table 1 Step elution from BN-cellulose of ³H counts from preparations of irradiated or unirradiated *E. coli*

<i>E. coli</i> strain	Strain ref.	Pertinent genotype	UV irradiation 6.0 J m ⁻²	Reincubation 50 min	% Recovered counts eluting with	
					No caffeine	2% Caffeine
(K) AB3063	(13)	<i>uvrA</i> EndoI	—	—	95	5 ± 1
			+	—	40	60 ± 5
			+	+	88	12 ± 3
(K) AB2487	(3)	<i>recA13</i>	—	—	87	13 ± 1
			+	—	60	40 ± 6
			+	+	84	16 ± 1
(B) WP2	(9)		—	—	93	7 ± 1
			+	—	67	33 ± 2
			+	+	95	5 ± 2
(B) WP5	(9)	<i>lex</i>	—	—	86	14 ± 6
			+	—	70	30 ± 5
			+	+	92	8 ± 2

in gap filling in leaky mutants, a 60 min reincubation should permit significant gap closure, measurable on alkaline sucrose gradients, which is not observed³.

Studies by Witkin⁹ and others suggest that some *E. coli recA* mutant cells can survive low doses of UV but are differentially repaired with less error than *recA*⁺ survivors. Presumably some completed gap filling occurs after a 24 h reincubation⁹. The identity of proteins responsible for this completion of gap filling or final closure and the fate of the pyrimidine dimer during error-prone repair are now under investigation in this laboratory.

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Repair of DNA damaged by alkylating carcinogens is defective in xeroderma pigmentosum-derived fibroblasts

THE human hereditary skin disease xeroderma pigmentosum (XP) is characterised by extreme sensitivity to sunlight and a high incidence of sunlight-induced skin cancer. These symptoms seem to be correlated with a defect in the excision repair of ultraviolet-induced dimers¹. Repair of DNA damage caused by chemical carcinogens belonging to the group of monofunctional alkylating agents was thought to be normal in XP cells, however, because after treatment with these agents the amounts of repair replication^{2,3} and the numbers of chromosome aberrations⁴ were similar in normal and XP cells. Contrary to this indirect evidence, it is reported here that direct measurement of the amounts of two products formed by alkylating carcinogens in the DNA and of the rate at which they are eliminated indicates that XP cells have a defect in this type of repair also.

XP-derived fibroblasts and normal human fibroblasts were treated with the potent carcinogens *N*-methyl-*N*-nitrosourea (MNU) and *N*-ethyl-*N*-nitrosourea (ENU). From the alkylation products formed, only the amounts of 7-alkylguanine and *O*⁶-alkylguanine were measured—7-alkylguanine because it is a major product and indicates the overall reactivity of the carcinogen with nucleophilic centres in the DNA and *O*⁶-alkylguanine because it seems to be critical for the mutagenic and carcinogenic action of alkylating agents⁵⁻⁹. *O*⁶-alkylguanine has been shown to cause mis-coding and anomalous base pairing⁵, and the relative amounts of this base that are produced by different agents seem to correspond to their carcinogenic potency⁶. In addition, the inability of an organ to excise *O*⁶-alkylguanine enzymatically from its DNA correlates with the organ-specific carcinogenic action of alkylating agents⁷⁻⁹.

Human fibroblasts from a normal donor (GM637) and from an XP patient of complementation group A (XP12RO) were treated with 8.7 $\mu\text{g ml}^{-1}$ ³H-MNU or 100 $\mu\text{g ml}^{-1}$ 1-¹⁴C-ENU for 45 min. The DNA was isolated 0, 24 and 48 h after the treatment and, after DNA hydrolysis, the purine bases were separated by Sephadex G10 chromatography (see Table 1 and ref. 10). The amount of each alkylguanine was determined from the radioactivity eluted in the position of the appropriate marker and was expressed as a fraction of the total guanine.

Normal and XP cells had about the same amounts of alkylated guanine immediately after treatment with an alkylating agent (Table 1), and the relative yields of *O*⁶-alkylguanine and 7-alkylguanine corresponded to those found *in vivo* in the rat^{10,11} and *in vitro* after reaction of DNA with MNU or ENU (refs 6, 10). The loss of 7-alkylguanine from DNA was similar in both cell lines, but there was a significant difference in the loss of *O*⁶-alkylguanine. Whereas in normal cells *O*⁶-alkylguanine was reduced to 28% of the initial amount 48 h after MNU treatment and to 9% 48 h after ENU treatment, there were still 63% (MNU) and 70% (ENU) of the initial *O*⁶-alkylguanine in XP cells at this time.

These reductions of alkylguanines partly reflect dilution by cell proliferation because the amount of alkylguanine is expressed as a fraction of the total guanine. To exclude this dilution effect and to eliminate differences in the rate of cell proliferation between cell lines, *O*⁶-alkylguanine was expressed as a fraction of 7-alkylguanine (Fig. 1). In normal cells this ratio decreased to one-third of the initial value 48 h after treatment with ENU and stayed constant after treatment with MNU, indicating the *O*⁶-alkylguanine was lost from the DNA as fast as or faster than 7-alkylguanine. In XP cells, however, the ratio of the two agents about doubled during the 48 h for both ENU- and MNU-treated cells, which means that 7-alkylguanine was lost much faster than *O*⁶-alkylguanine in these cells.

The reason for the similar rates of loss of 7-alkylguanine in normal and XP cells but the very different rates of loss of *O*⁶-alkylguanine may involve different mechanisms for removing the two bases. Alkylation of guanine at the N7 position labilises the *N*-glycosidic bond and leads to non-enzymic depurination of the base¹². A mechanism of 7-alkylguanine loss based on spontaneous depurination and dilution by cell proliferation predicts that similar rates of loss would occur in cells that have similar division rates. This was the case in normal and XP cells (Table 1), although enzymic excision cannot be excluded. *O*⁶-alkyl-

Fig. 1 Ratio of *O*⁶-alkylguanine to 7-alkylguanine in the DNA of normal human fibroblasts (---) and XP fibroblasts (—) after treatment with methylnitrosourea (○) or ethylnitrosourea (▲) as a function of time.

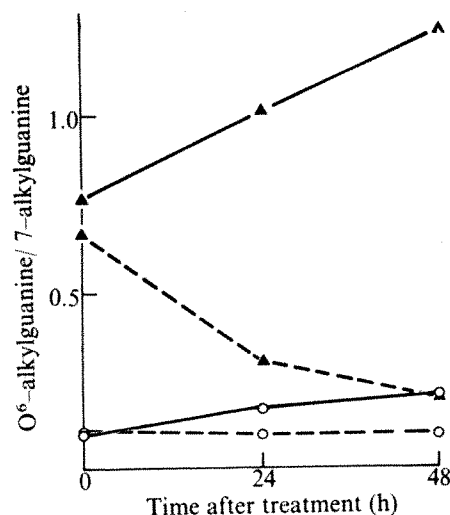


Table 1 Amount of alkylated guanine in the DNA of normal and xeroderma pigmentosum fibroblasts after treatment of the cells with ^3H -methylnitrosourea or $1\text{-}^{14}\text{C}$ -ethylnitrosourea as a function of time

Cell type	Time after treatment (h)	^3H -methylnitrosourea ($8.5 \times 10^{-5} \text{ M}$)				$1\text{-}^{14}\text{C}$ -ethylnitrosourea ($8.5 \times 10^{-4} \text{ M}$)			
		7-Methyl-guanine ($\times 10^6$)	% of 0 h value	O^6 -Methyl-guanine ($\times 10^6$)	% of 0 h value	7-Ethyl-guanine ($\times 10^6$)	% of 0 h value	O^6 -Ethyl-guanine ($\times 10^6$)	% of 0 h value
GM637	0	181	—	19	—	31	—	21	—
	24	106	59	10	53	17	55	5	24
	48	51	28	5	28	9	30	2	9
XP12RO	0	169	—	16	—	30	—	23	—
	24	78	46	14	88	16	53	16	70
	48	48	28	10	63	13	43	16	70

Cell lines, which had been transformed by SV40 virus, were chosen because of their high rate of cell division. Previous studies have demonstrated that transformation does not alter DNA repair characteristics of human fibroblasts^{21,22}. The cells were grown in Eagle's minimum essential medium with 10% calf serum in 1-gallon roller bottles. For one alkylation experiment cells from four confluent cultures were trypsinised and resuspended in 25 ml of serum-free Eagle's minimum essential medium in a Spinner flask and stirred at 37 °C at a speed that kept cells in suspension. The alkylating agent was then added— $8.7 \mu\text{g ml}^{-1}$ ^3H -MNU ($123.2 \text{ Ci mol}^{-1}$, 0.87 mg ml^{-1} in ethanol) or $100 \mu\text{g ml}^{-1}$ $1\text{-}^{14}\text{C}$ -ENU (4.98 Ci mol^{-1} , dissolved just before use in citric acid-disodium phosphate buffer, pH 6)—and the cells were incubated for 45 min. DNA from one-third of the cells was isolated immediately. The rest of the cells were put back into roller bottles and grown for 24 or 48 h before collection by trypsinisation. Cells were lysed in 0.1% sodium dodecylsulphate in SSC (0.15 M sodium chloride + 0.015 sodium citrate) and then incubated first with RNase (final concentration 0.1 mg ml^{-1}) for 1 h and then with pronase (final concentration 1 mg ml^{-1}) for 2 h at 37 °C. The DNA was precipitated by 2 volumes of ethanol and hydrolysed in 0.1 N HCl (37 °C, 20 h); the purine bases were separated by chromatography on a Sephadex G10 column ($80 \times 1.5 \text{ cm}$) eluted with 0.05 M ammonium formate (pH 6.8). The amount of each alkylated guanine was calculated from the specific radioactivity of the given alkylating agent and the integral radioactivity of the chromatographic peak corresponding to a marker for the appropriate alkylated base, and was expressed as a fraction of the total guanine in the isolated DNA, which was determined from the absorbance of the guanine fractions from the column eluates. For details see ref. 10.

guanine, on the other hand, is chemically stable in DNA^{10,13} and must be enzymically excised. Because the XP cells under investigation lost much less O^6 -alkylguanine than normal cells did, this loss might have been completely due to dilution by proliferation rather than to excision.

Although the doses of MNU and ENU used in this experiment produced almost the same amount of O^6 -alkylguanine, the observed excision from the DNA of normal cells was much faster after ENU treatment than after MNU treatment (Table 1, Fig. 1). This might imply that the ethylated derivative is excised more efficiently. But, MNU also produced much higher overall alkylation in the cell, the alkylation at the N7 position of guanine being, at the doses used, about six times higher than with ENU, so a similarly higher reaction with proteins can be expected. Thus, a reasonable explanation for the slower excision of O^6 -methylguanine is that enzymes of the repair system are partially inactivated by the MNU dose used.

Though it has been shown that O^6 -alkylguanine is excised enzymically in mammalian cells, it is not yet known if the enzyme is of the nucleotidase type or of the N-glycosidase type¹⁴. The enzyme that excises O^6 -alkylguanine in *Escherichia coli*, endonuclease II¹⁵, seems to be an N-glycosidase that works in conjunction with an apurinic endonuclease¹⁶. If the same mechanism is at work in mammalian cells, then XP cells of complementation groups A and D may have a defect in the entire repair process, because it has already been shown that there is a defect in their apurinic endonuclease activity¹⁷.

This type of repair seems to have an important role in carcinogenesis. It has been shown that the rate at which O^6 -alkylguanine is excised varies greatly in different rat tissues, being fastest in liver, intermediate in kidney, and very slow in brain⁷⁻⁹. As MNU and ENU are both nervous-system-specific carcinogens in the rat¹⁸, the inability of the brain to excise this potentially mutagenic damage correlates with the organ-specific carcinogenic action of these agents. The inability of XP-derived fibroblasts to excise this damage might not be representative of all XP-derived cell types, although it seems unlikely that the inheritable change connected with this disease would be expressed in only one cell type.

It has recently been reported that, unlike the number of chromosome aberrations, the frequency of sister chromatid exchanges is much higher in XP cells than in normal cells

after treatment with alkylating agents¹⁹. This raises the question of whether there is a causal relation between the unexcised O^6 -alkylguanine and the increased sister chromatid exchange.

Because measurements of repair replication by isopycnic analysis and by autoradiography after treatment with alkylating agents do not show a difference in repair by normal and XP cells^{2,3}, these techniques do not seem applicable when various types of damage occur. It especially does not seem to be an accurate measure of excision, because the main product, 7-alkylguanine, depurinates spontaneously. Also, although almost no repair is detectable by this technique 20 h after ENU treatment of normal cells²⁰, the work described here shows that more than 50% of the alkylated bases are still present in their DNA.

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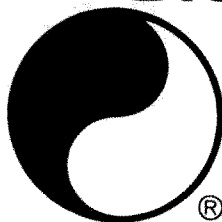
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reviews

Algal genetics

Lynn Margulis

The Genetics of Algae. Edited by Ralph A. Lewin. Pp. viii+360. (Blackwell Scientific: Oxford and London, 1976.) £11.50.

THIS book is a beautifully edited and profound review of the state of genetics of algae, which, as Lewin says, "are more of a guild than a clan, united more by common aptitude than by common ancestry". Although all algae have been taken to be within the province of the work, in fact there is very little serious genetics on organisms other than the green algal genus *Chlamydomonas*.

There are thirteen separately authored sections in the book. These include reviews of the recently established field of the genetics of blue green algae, the formal (mainly nuclear, mendelian) genetics of *Chlamydomonas reinhardtii*, genetic determinants of flagella phenotype in *C. reinhardtii* (a frustrating subject to study since flagellar alterations in these organisms which mate 'flagella first', tend to impair the frequency of viable crosses) and a short chapter on the genetics of cell wall formation in *C. reinhardtii*. The cell wall summary not only indicates how useful mutational analysis ultimately may be in the deciphering of the complexities, chemical and ultrastructural of cell wall morphogenesis, but indicates that there may be still another enigmatic "non-mendelian genetic system" in this chlorophyte.

C. reinhardtii, of course, is the star actor in any eukaryotic cell genetics drama and this is aptly indicated in the two chapters by Gillham, Boynton and their colleagues on plastid inheritance and genetic control of ribosomal biogenesis. The relationship of the three genetic systems (nuclear, plastid and mitochondrial) has nowhere been better investigated than in this rapidly growing isogamous organism, which may be mated at will, completes its life cycle in about a week and for which gene dosage and genotype-phenotype relationships can be studied since stable diploids can be made.

Information concerning the genetic systems of *C. moewusii* and *C. eugametos*—two other species of *Chlamydomonas* rivalling *C. reinhardtii* for centre stage—is reviewed. Since

stable heterokaryons can be made in *C. moewusii*, complementation can be studied in this organism. The reason why 2 amino-3 butanoic acid induces both gene mutation and chromosomal aberration in *C. eugametos* is still obscure. No such effect has been seen in *C. reinhardtii*. Other differences between the three species have been noted as well, providing internal tests for the possible generalisation of observations made on *C. reinhardtii*.

Although an exciting comprehensive, and up-to-date treatise, this book makes us acutely aware of how limited our genetic knowledge is concerning the algae as a group. There literally is no genetic literature as such on the red algae, the brown algae, the diatoms, the dinoflagellates, the chrysophytes, the prasinophytes, the euglenids, the haptophytes, and so forth. Although there is some scattered information concerning the mating and differentiating systems in *Volvox*, conjugating green algae, 'multicellular marine algae' and *Acetabularia* in chapters on the genetics of the Volvocales, Zygnematales, and Charophyta, respectively, even these sexual green algae have managed to hold on to their genetic secrets.

The classical techniques of controlling the life-cycle of marked genetic stocks, crossing individuals of opposite mating types and measuring the distribution of traits in offspring of these crosses are simply not available in the vast majority of algae for several different reasons. For example, the genetics of an organism such as *Euglena gracilis*, a flagellate which has yielded significant information concerning mitochondria and chloroplast inheritance and development, is impossible to study. Although chloroplast development and differentiation can be induced, these fast growing single cells simply refuse to mate. Thus, all of these chapters referred to above are forced to deal with "genetically related" phenomena (for example, hybrids produced by grafting in *Acetabularia*, nucleic acids of organelles).

Furthermore, the book is overwhelmingly concerned with the eukaryotic green algae: the Chlorophyta. As for the Cyanophyta or blue greens, they are a world apart. True to their popular new name (Cyanobacteria) they are

only rudimentarily sexual, anything at all about genetics is known in very few species. In general, they rely on proven prokaryotic mechanisms for gene transfer—transformation and transduction—in so far as any occurs. One new realisation is the nature of the circular genetic map as deduced from mutagenesis studies in synchronous cultures of *Anacystis nidulans*, a coccoid blue green: it really resembles in overall features, the genetic map of *Escherichia coli*.

Some idiosyncratic but profound algal genetic anecdotes come to light in this well illustrated and produced book. For example: in some blue greens recombination seems to be regularly accompanied by mutation; in *Ulva* the chromosomes are apparently composed of four rather than two DNA strands, and in *C. eugametos* a specific mutator gene reverses mutations at two nicotinamide loci.

Probably the most exciting advances concern the unravelling of biochemical interactions between the three classes of genetic organelles in *C. reinhardtii*: nucleus, mitochondria and plastids. Indeed sex in organelles exists and genetic maps have been made of a cytoplasmic genetic system. (Presumably these maps are of the chloroplast linkage groups: recombination of plastid DNA has been discovered.) There is even some evidence that a single mutation may alter both chloroplast and mitochondrial ribosomes simultaneously. Several chloroplast ribosomal proteins are apparently synthesised on cytoplasmic ribosomes even though the chloroplast DNA probably codes for all the chloroplast RNA.

The book is full of such well explained surprising genetic phenomena, and is highly recommended for advanced genetic and phycology courses and the professional reader. *The Genetics of Algae* is indispensable to those interested in genetic systems of the 'lower eukaryotes'. The book is well written, comprehensive and comprehensible. If more work on the other fascinating algal groups has not been reported here, it is because it has not yet been done. □

Lynn Margulis, Professor in the Department of Biology at Boston University, is on leave as Sherman Fairchild Distinguished Scholar at the California Institute of Technology, Pasadena.

Aggregation chimaeras

Mammalian Chimaeras. By A. McLaren. Pp. 154. (Cambridge University: Cambridge, London and New York, 1976.) £8.

It might be said that the laboratory mouse is the *Escherichia coli* of modern mammalian biology, since great things are expected of this species. Mouse genetics is one field in which the Woman's Liberation Movement is already passé, for the field has long been dominated by a group of outstanding ladies. Dr McLaren is certainly one of them. Her little book, as she lovingly calls it, certainly shows her intelligence and breadth of understanding.

Characteristically feminine attentions to details are most pleasing. The subjects are mostly what have variously been called allophenic or tetraparental mice produced by the fusion of two blastocysts, but Dr McLaren prefers to call them aggregation chimaeras. Injection chimaeras refer to those that are produced by the injection of one or more cells into a blastocyst of a different genotype as practiced by Richard Gardner. These animals are very useful for analysis of clonal differentiation of embryonic cells. Degrees of intermingling that inevitably occur between neighbouring clones can also be assessed. They are also ideally suited to distinguish genetic effects that are mediated through humoral factors from those that are

exclusively intracellular. The wealth of information accumulated by Tarowski, Minz, McLaren, and by a number of others, is presented in a very orderly manner and in sufficient detail.

Inevitably, a few very important pieces of information apparently failed to make the deadline, but who can blame the author for this. For example, M. F. Lyon's recent demonstration that totally androgen-insensitive *Tfm/Y* male germ cells in the body of chimaeric male mice can mature and differentiate to functional spermatozoa was nearly a fatal blow to those who believed in the androgen dependence of male germ cells. Similarly, the discussion on the great preponderance of males among XY/XX chimaeric mice would have become more meaningful if more recent findings on H-Y antigen's testis-organising functions were introduced.

With regard to immunology, another recent finding that Ia antigens, controlled by at least two immune response (Ir) loci, are very strongly expressed on B cells, but only very weakly on most T cells, would surely have modified Dr McLaren's discussion of findings on chimaeric mice between high responder and low responder strains.

I believe that almost every problem-solving biologist who deals with higher vertebrates would find this book instructive and informative.

Susumu Ohno

Susumu Ohno is Chairman of the Division of Biology, City of Hope National Medical Center, Duarte, California.

Practical problems of the visual world

Vision and Acquisition: Fundamentals of Human Visual Performance, Environmental Influences and Applications in Instrumental Optics. By Ian Overington. Pp. xix 380. (Pentech: London; Crane, Russak: New York, 1976.) £14.

THIS is an unusual and useful book. Its main value is that it focuses attention on the problem of how a human detects and recognises a target in adverse conditions.

Although much of the work described was undertaken for military purposes, many of the results are pertinent to civilian life—for example, the flying of modern high speed aircraft poses many human problems particularly in visual performance. But even at the lower speed of driving a car many accidents are believed to occur because of a failure in visual perception or recognition. This is

particularly true in motorway driving in poor visibility, often resulting in a tragic loss of life and injury.

The book is particularly valuable in that it contains many references to special reports that most visual scientists would not come across. There are a few errors particularly when the physiology of vision is discussed, but none of these are of a misleading nature.

I hope that this book will encourage some laboratory bound visionaries to look out of the window and consider the practical problems that the visual world presents. Conversely, it may also encourage engineers to pay more attention to modern visual physiology and possibly even to seek the advice of visual scientists more frequently.

The book is very neatly presented and illustrated, and bears the high price of today's publication costs.

F. W. Campbell

F. W. Campbell is Reader in the Department of Physiology at the University of Cambridge, UK.

Virology symposia

Animal Virology. (ICN-UCLA Symposia on Molecular and Cellular Biology. Vol. IV. Edited by D. Baltimore, A. S. Huang and C. F. Fox. Pp. xi+824. Academic: New York and London, 1976.) \$29.50; £18.

THE ICN-UCLA Symposia on Molecular and Cellular Biology held each spring at skiing resorts in California or Colorado provide a pleasant and useful forum for the subjects discussed. Baltimore, Huang and Fox organised what seems to have been a most interesting meeting on animal viruses, that even included a session on plant viruses. Much of the emphasis was on viruses known to be oncogenic, including an interesting session on cell transformation and differentiation, but there were also sessions on the molecular organisation of negative-strand RNA viruses, on viral messenger RNA synthesis, and on viral protein synthesis. Thus, as the organisers say in the preface (as editors of the published proceedings), "this meeting considered broad issues and recent advances in animal virology, and treated tumor viruses in the context of the range of animal viruses rather than as a separate problem."

In my opinion this volume should never have been published. True, it is one of the better volumes of its kind, but I deplore the practice of routine publication of symposia proceedings. It clutters up the literature and overburdens library budgets. Perhaps some of these volumes are profitable to publishing companies: they are not profitable to scientific endeavour. The only purpose it serves is to amplify the contributors' publication lists. The ICN-UCLA symposia are published by typescript photo-reproduction methods and appear about 6 months after the meetings are held. Most of the papers in the volume under review had by that time been printed with essentially identical data in academic journals as proper publications. Those that have not, may have been disapproved by reviewers and editors. The symposia proceedings are not subject to review and there is no distinction between invited and proffered papers.

Scientific organisers of symposia are usually apologetic on requesting manuscripts for publication, explaining that the financial sponsors demand them. The sponsors should realise that the meeting is important, not the publication, and there are other ways of making the sponsorship known. It is time that scientists declined to present talks on current research at symposia if publication is obligatory.

Robin Weiss

Robin Weiss is a member of staff at the Imperial Cancer Research Fund Laboratories, London, UK.

Bioengineering

Applied Biochemistry and Bioengineering. Vol. 1: Immobilized Enzyme Principles. Edited by L. B. Wingard, Jr, E. Katchalski-Katzir and L. Goldstein. Pp. xi+364. (Academic: New York and London, 1976.) \$29.50; £20.95.

RECENTLY there has been a spate of books on bioengineering in general and enzyme technology in particular. Many are collections of unrelated articles of very variable quality. Therefore it is pleasant to note that this first volume of a new series has a coherent theme which is well treated. The editors do have a particular reason to organise the volume well. Wingard has done much to stimulate interest in enzyme technology, and Katchalski-Katzir and Goldstein were pioneers in the fundamental studies of immobilised enzymes. Appropriately the latter two editors begin the book with a concise survey of developments in the field which serves to introduce subsequent contributors. Goldstein is also co-author (with Manecke) for the second chapter on the chemistry of enzyme immobilisation. The systematic treatment of the available methods with comment on their advantages and disadvantages will be welcomed by anyone who has to make a selection from the bewildering number now available.

Engasser and Horvath deal with diffusion and kinetics of immobilised enzymes.

Synaptic connections

Neuronal Recognition. By S. H. Barondes. Pp. xvi+367. (Plenum: New York and London, 1976.) \$33.

THIS collection of eleven essays is concerned with the problem of how neurones establish synaptic connections with certain targets and not with others. As a result of studies on the developing and regenerating nervous system, we now have a reasonable picture of the degree of discrimination in several situations, but not of the underlying cellular and molecular mechanisms. The essays vary from relatively general reviews to quite detailed accounts of work from the authors' laboratories. Together, they provide a good overall view of approaches to the problem.

The existence of specificity, as well as its extent and possible basis, is considered for the retinotectal system (Jacobson), for the neuromuscular junction (Fambrough), for sprouting in the central nervous system (Cotman and Lynch), and for synaptogenesis in tissue culture (Bunge). Morphological information about early events in synapse formation is considered by Pfenninger and Rees, and the current unsatisfactory state of biochemical methods for purifying and

They draw heavily on the results of their collaborative studies in dealing, for example, with the effect of external diffusion limitation on the observed behaviour of enzymes immobilised in porous particles or membranes.

The review of the design and analysis of immobilised enzyme flow reactors by Vieth *et al.* is well documented but the reviewer would arrive at some different conclusions. The authors state that 'batch reactors have limited potential in industrial immobilised-enzyme catalysis.' Most of the semisynthetic penicillins produced industrially in Europe and a growing proportion elsewhere are made by means of batch conversion using immobilised enzyme.

The industrial application of immobilised enzymes and immobilised microbial cells are described with technical and economic details by Chibata and Tosa, whose Japanese company was the first to announce the use of both types of catalyst in modern industrial processes. It is interesting that glucose isomerisation, which originated in Japan but was developed as the largest-scale current use of immobilised cells or enzymes in the USA, is not dealt with in any detail, presumably because it does not have the same economic importance in Japan. **P. Dunnill**

P. Dunnill is Lecturer in Biochemical Engineering and co-leader of the enzyme and protein technology group in the Department of Chemical and Biochemical Engineering at University College, London, UK.

fractionating neural plasma membranes is critically reviewed by Morgan and Gombos.

The second half of the book is concerned with attempts to assay and identify molecules involved in cell recognition in various situations. Although there is, as yet, no direct evidence on the relevance of these studies to synapse formation, they are clearly of great interest to students of specificity. Three of these essays are concerned with investigations of cellular aggregation and adhesion in the retinal and retinotectal systems (Moscona; Roth and Marchase; Merrell, Gottlieb and Glaser). The morphogenetic role of extracellular glycosaminoglycans is considered by Toole, and Barondes and Rosen discuss cell surface lectins and cell recognition in slime moulds.

Most of the essays are thoughtful, well organised and often helpfully illustrated. Neurobiology sometimes seems impenetrable to outsiders, but I would strongly recommend this volume to interested investigators in other areas of cellular and molecular biology, as well as to graduate students and specialists in the field. **Jeremy Brockes**

Jeremy Brockes is a member of the MRC Neuroimmunology Project at the Department of Zoology, University College, London, UK.

Fourier transform NMR spectroscopy

Fourier Transform NMR Spectroscopy. By D. Shaw. Pp. xvi+357. (Elsevier Scientific: Amsterdam, Oxford and New York, 1976.) Dfl. 129; \$49.75.

FOR a great deal of chemical structural and diagnostic work using proton nuclear magnetic resonance (NMR), a simple continuous wave (CW) spectrometer is entirely adequate, but in cases involving very small molar quantities of sample or for measurements on other nuclei, Fourier transform (FT) spectrometers are needed. The CW spectrometer scans slowly across the spectrum, using a continuous monochromatic radio frequency to stimulate each resonance in turn. In the FT spectrometer, a brief but strong pulse of radio frequency is applied to the sample so as to stimulate all the resonances simultaneously. The response of the nuclear spins after the pulse is collected, and because the components of all the resonances are mixed up together (and in the time domain) a computer is needed to disentangle them and produce the more familiar spectrum (in the frequency domain). Because all the resonances are stimulated simultaneously, there is a substantial gain of signal-to-noise for complex spectra, and because the method uses pulsed radio frequencies, relaxation times are more easily measured than with the CW method.

Dr Shaw has produced an excellent handbook for those spectroscopists who are extending their activities from the CW to FT methods. In chapter 3 the mathematical principles are outlined, not in an attempt to provide rigorous accounts of the theorems, but to ensure that the physical principles are clear and the equations used can be understood. Chapters 4 and 5 give a clear description of the various ways of exciting NMR spectra and their relative advantages; and chapters 6 and 7 explain in some detail the experimental methods available, the equipment required, how it works, and the ways in which it may be used. These chapters form the core of the book and will be of great value to all those who need to use FT spectrometers.

Chapters 1 and 2 give a brief introduction to the principles of NMR spectroscopy; and chapters 8, 9, 10 summarise many of the ways in which NMR spectroscopy can be applied to chemical problems and how the measurable parameters can be obtained. These chapters cover a lot of ground and are useful mainly as a convenient outline and reminder to readers already acquainted with the subject. **R. E. Richards**

R. E. Richards is Warden of Merton College, Oxford, UK.

Machiavellian weed

Wild Oats in World Agriculture: An Interpretative Review of World Literature. Edited by D. P. Jones. Pp. xi+296. (Agricultural Research Council: London, 1976.) £3.

It is unusual, if not unique, for a weed to achieve the distinction of having such a comprehensive publication devoted to itself, an accolade most often accorded to a crop species. The impetus for a review at this time has been dictated primarily by the requirements of agriculture for greater understanding of the biology and control of this pernicious species. It is perhaps Joan Thurston at Rothamsted, however, who must be acknowledged as having foreseen in the post-war period the emergence of this species as a primary constraint to the cereal producer and initiated much needed research and the collection of material from throughout the world, on which so much of the information on distribution of *Avena fatua* is based.

The significant contribution to the systematic appraisal of the biology and agronomy of wild oats by the Weed Research Organisation in the last decade is reflected in the authorship of this book. A leavening of the text, however, with some contribution from outside the UK, would have been useful in consolidating the purported world aspect of the review. Research in Canada with respect to the fundamental mechanisms of seed dormancy and at a more general agronomic level has been considerable. The whole nature of the problem in North America, with its extensive low input cereal production system, stands in stark contrast to the situation in Western Europe: the role of Government and statutory controls could also have been treated in a somewhat wider perspective.

A wealth of information has been gathered together in this volume. In this topic, there undoubtedly exists the basis of a classic contribution, not just to agriculture or botany but of relevance to many areas within the broad biological discipline. There are so many elements in the biology of the wild oat of wide and general significance—a weed par excellence and one whose resources and versatility, strengths and weaknesses, have been so well documented to exemplify the myriad facets of a successful weed—that it is to be hoped that this volume will achieve a circulation outside that of the specialist agriculturalist for whom it was mainly intended. The mechanisms of seed

dormancy and seed dissemination, the population dynamics in the context of agricultural practice, the niceties of selective herbicide usage which now allow a distinction to be drawn between the 'wild' and cultivated varieties of oats, are examples of situations of wide biological relevance.

My prime reservation is that the authors have aimed this work at a specialist readership, whereas the subject has a great deal of popular appeal and that the fascinations of this Machiavellian weed have been subjugated, if not totally submerged, by the bulk of references and verbiage.

On a more specific level, although the chapters follow a logical sequence there are incongruous aspects to the

section and subsection headings in certain chapters. A degree of unnecessary duplication of material between topics is also to be found although, with the exception of minor omissions in pagination, the difficult task of adequate cross referencing has been well accomplished.

In all, this volume stands as a novel concept within, and a valuable contribution to, the field of the biology and control of weeds, and at such a modest price represents exceptional value.

Alan D. Courtney

Alan Courtney is Lecturer in the Department of Agricultural Botany at the Queen's University, Belfast, Northern Ireland.

Laser applications

CO₂ Lasers: Effects and Applications. By W. W. Duley. Pp. xiii+427. (Academic: New York, San Francisco and London, 1976.) \$31; £18.40.

THE preface to this book is more informative than its title: the volume outlines a very wide range of laser applications in physics, chemistry and engineering, with the CO₂ laser receiving special rather than exclusive attention. In places the treatment is distinctly pragmatic. For example, in the forty or so pages dealing specifically with CO₂ laser devices, optimisation of optical cavity transmission is treated entirely experimentally, with no discussion of Rigrod's formulation. A second example is provided in the surprisingly brief treatment of unstable optical resonators, where there is no interpretation of the specific experiments cited.

Readers may feel that chapter 3 redeems itself with the very detailed information provided on infrared detectors, windows, modulators and mirrors, although diamond turning techniques receive no mention. The first half of the book concludes with selected solutions to the heat-conduction equation, a subject previously, exhaustively and authoritatively treated by Carslaw and Jaeger. Moving heat sources, liquid phases and vaporisation do, however, provide interesting variations to this theme, and are required for a proper understanding of surface heat treatment and welding; here, an important reference (Swift-Hook and Glick, 1973) has been inadvertently omitted in the references.

An interesting survey of drilling by argon, ruby, Nd-YAG and CO₂ lasers is followed, in chapter 6, by a somewhat dated discussion of welding and machining. In particular, Table 6.5

contains several errors and omits any specification of spatial mode quality of the laser, or of focal conditions—factors which may account for some of the relatively poor cutting speeds tabulated. The laser-induced reactions, evaporation and thermal effects touched on in chapters 7–9 are uniformly intriguing, and could interest a diverse selection of *Nature's* readership. These applications include: laser cleansing of surfaces, the deposition of thin films, chemical vapour deposition, surface studies and reactions, geological analyses, laser-triggered spark gaps, resistor-trimming, pyrolysis of coal and oil shale, gas-phase reactions and spectroscopy, crystal growth, metallurgical effects, and controlled stress generation in solids and liquids. This coverage is clearly too wide to permit detailed evaluation of specific topics; in particular, isotope separation and (thermal) surface heat treatment are both treated too briefly to be of interest to specialists in these fields. The book concludes with a chapter discussing propagation, atmospheric monitoring and the communications possibilities of the CO₂ laser. Pollution monitoring to parts in 10⁻⁹–10⁻⁸ using tunable diodes, spin-flip CO, CO and CO₂ lasers, and the back-scattering efficiency for typical particulate atmospheric constituents, are discussed in some detail. A systematic comparison of LIDAR performance using a range of laser sources is however, not attempted. Three appendices provide a useful compendium of thermal constants for a range of engineering materials.

The book is well produced, reasonably priced, and provides an interesting introduction and bibliography to the scientific and technical literature.

I. J. Spalding

I. J. Spalding works on laser applications at the UKAEA Culham Laboratory, Abingdon, UK.

Extraversion-introversion paradigm

The Measurement of Personality. By H. J. Eysenck. Pp. xviii+511. (MTP: Lancaster, 1976.) £12.50.

EYSENCK is remarkable in many ways, not least in his ability to generate books both as author and editor. This one, a collection of reprints, linked tightly and at length by editorial argument, deals almost entirely with the personality dimension of extraversion/introversion but has no overlap with the papers covered by his recent three-volume work on the same topic.

Nevertheless, the papers are carefully selected to exemplify and support the case for Eysenck's particular approach to the study of personality which he describes as a 'paradigm' in the Kuhnian sense of a professional or disciplinary matrix. This paradigm is closely modelled on physical science, depends on measurement and hypothetico-deductive procedures but goes beyond Popper to Lakatos' view of a research program which yields predictions of novel facts but tolerates anomalies among its findings; and in fact progresses from experimental analyses of these anomalies.

Eysenck's program is based on the claims that there is a set of invariant general traits underlying individual differences in behaviour, that these are explicable in terms derived from general experimental psychology, and that the broader and more important personality factors are likely to derive from more basic biological variables which, in turn, are largely determined by genetic factors. Apart from intellectual abilities he believes that there are only three higher order factors of this type. Extraversion/introversion (E) is one of these and the related behaviour is postulated to be a non-linear function of individual differences in characteristic levels of "cortical arousal". Sections of the book deal with models of personality, its physiological basis, differential reactions to pain and other sensations, and individual differences on vigilance tasks, in perceptual reactions, motor behaviour, learning, memory, fluency and creativity, and in social behaviour.

Many of Eysenck's opponents consider his objective, nomothetic approach inappropriate for the study of personality and engage in a holistic idiographic search for understanding, which he sees as fruitless. Others remain within his frame of reference but lay greater stress on the situational determinants of behaviour, which he

regards as largely irrelevant or as interfering factors to be controlled in his studies. He does recognise that they may have an important function in certain real life situations but even then stresses their interactions with an individual's personality. Other critics also quarrel with the heavy weighting Eysenck grants to heredity in his rather "either/or" approach to nature/nurture questions. A particular phenotype is the outcome of a developmental process in which experience may affect organismic maturation in a number of ways: by channelling it in certain directions but also by facilitating or inhibiting normal development. This is particularly true of early neural maturation, which plays an important role in Eysenck's theory.

The great strengths of Eysenck's personality program, however, are that it embraces more levels of functioning—biological, behavioural and social—in a more comprehensive way than any other and that it is closely related in an interactive developing fashion with an ongoing experimental research programme. In this book he certainly substantiates his claim to a "paradigm" and a theory in transition from "soft" to "hard". His critics should read this book with care: they will not find it easy to produce an equally powerful counter-argument.

H. Gwynne Jones

H. Gwynne Jones is Professor of Psychology at the University of Leeds, UK.

Solar flares

Solar Flares. By Z. Svestka. Pp. xiv+399. (D. Reidel: Dordrecht, The Netherlands and Boston, Massachusetts, 1976.) Cloth Dfl115; \$39.50; paper Dfl69; \$23.

THERE may be a temptation to regard Svestka's book as an update of Smith and Smith's work of the same title published fourteen years ago. To do so would, however, be to grossly underestimate the magnitude and success of Svestka's undertaking in this new book. For, firstly, the quantity of observational material to be reviewed has expanded since 1963 with a growth rate perhaps exceeded only by solar flares themselves. And, secondly, although the subject matter is intended primarily to be observational, an admirable awareness has been retained throughout of the need for objective interpretation in the areas of both classification and physical theory.

Approximately one-third of Svestka's efforts are devoted to the chromospheric flare as observed in optical wavelengths, and to flare-associated optical phenomena. Although this distribution may still reflect some of the historical overemphasis of hydrogen Balmer line observations, it seems well justified in view of Svestka's personal expertise in this area, and, also of the observations being well tempered by theoretical discussion of such matters as inference of magnetic fields and models of chromospheric flare heating.

A comparable section is dedicated to "The High Temperature Flare" embracing not only the thermal XUV emissions but also the non-thermal hard X-ray and radio bursts. Much of this material appears here in mono-

graph form for the first time, having been born of the advances in space techniques over the past decade. The coverage given to Skylab and other recent results is remarkably good for a review of such a rapidly expanding field. Ample attention is again given to basic data interpretation and to detailed physical models where these exist—specifically for hard X-ray, microwave and Type III sources.

Following a chapter on particle emission, Svestka finally turns to the matter of flare models. In a sense, this is the weakest section since many different models are rather cursorily dealt with. This is not surprising since a second volume by the late L. D. de Feiter had been planned on purely theoretical aspects. Given, however, the current motley state of flare theory, and the complexity of the plasma physics involved, it is perhaps better simply to relate the broad features of models to observational manifestations rather than to attempt a survey of this difficult and incomplete area of theory.

Finally, it might have been appropriate to set the flare problem in a wider plasma astrophysical context such as theory of flare stars and energetic galactic phenomena. To do so would, however, have been to enter speculative realms not in keeping with the systematic objectivity which characterises this book and Svestka's work in general. This quality alone should suffice to remind astrophysicists of the intrinsic interest of the flare phenomenon, and certainly makes the book a must for all solar physicists.

J. C. Brown

J. C. Brown is Lecturer in the Department of Astronomy at the University of Glasgow, UK.

Biological effects of environmental agents

Biochemical Toxicology of Environmental Agents. By A. de Bruin. Pp. x + 1544. (Elsevier/North Holland: Amsterdam and New York, 1976.) Dfl. 320; \$130.75.

INTEREST in the biological effects of environmental agents has increased enormously in the past three decades, stimulated by the growing awareness of the potential threat to human health of the increasing number of chemicals in the environment. There has been a corresponding increase in the number of toxicological investigations (in man and experimental animals) and these have come to rely more and more on biochemical techniques both in the assessment of toxic effects and in the study of the mechanisms of toxicity.

Dr de Bruin's book provides in 42 chapters an up-to-date review of the many aspects of biochemical toxicology. The first chapters are fairly general in scope, dealing with the metabolism of foreign chemicals, with factors which modify metabolism and toxicity, and also with tests of exposure to toxic compounds. There follows a systematic treatment of the various aspects of toxicity which are considered in different chapters,

according to the biochemical or biological system which is affected. Thus, disorders of carbohydrate and lipid metabolism are first considered, then proteins, nucleic acids and so on, and, finally, immunological mechanisms and blood coagulation disorders. Each chapter has a short introduction outlining the normal biochemical principles relevant to the system, followed by the biochemical toxicology of chemical agents and the effect of ionising radiations.

Dr de Bruin's book will be a useful reference work. The amount of information presented is vast (about 13,000 references are quoted), and the book is unique in providing so much information on so many different aspects of biochemical toxicology in one single volume. That a single author should have been capable of covering so much ground on his own is in itself, a very considerable achievement.

Perhaps inevitably for a book of this size, treatment of the biochemical mechanisms of toxicity is sometimes superficial; the author presents all the data available in an exhaustive fashion with little critical appraisal, so that the reader is left without guidance as to which of the various findings reported are of particular significance.

F. De Matteis

F. De Matteis is a member of the scientific staff of the MRC Toxicology Unit, Carshalton, Surrey, UK.

Secrets of the Universe still locked up

The Universe. By J. Kleczek. Pp. vi + 259. (Reidel: Dordrecht and Boston, Massachusetts, 1976.) Cloth Dfl. 70; paper Dfl. 43.

THE idea of a book about the Universe linking the large and the small (galaxies and elementary particles), and starting from particle physics and the four basic forces to build outward, is a good one. Unfortunately, this is not a good book. The principle reason for this is the severe tailoring required to fit a quart story into a one-pint volume; as the preface says, this is only achieved by giving no references, short-cutting arguments, expressing quantities often as orders of magnitude and keeping formulae to a minimum. As well as all that, the author's English is not always very readable, and the text could have been improved by some sympathetic editing.

Of all these shortcomings, the worst is that where several theories or hypotheses exist to explain some pheno-

menon—as is so often the case in studies of the Universe—the author chooses only one for discussion, and presents it uncritically. On the solar neutrino problem, the author's treatment is even more deficient; we are simply told that "Many suggestions have been made to explain the discrepancy. There is no lack of ideas", without being told what any of the ideas are. The confused reader will gain little comfort from the index, in which some topics are broken down in nit-picking detail, whereas others receive no mention; the entry under "B", for example, consists solely of "Big-Bang", with no mention even of black holes, although they are discussed in the text.

If the same material, arranged under the same chapter and section headings, had been expanded to twice the size of the present book and edited into smoother English, the result would have been a valuable addition to the astrophysical literature. As it is, the present volume adds nothing of any value and cannot be recommended.

John Gribbin

John Gribbin is a member of the Science Policy Research Unit, University of Sussex, UK.

Ecophysiology

Introduction to Physiological Plant Ecology. By P. Bannister. Pp. 273. (Blackwell Scientific: Oxford and London, 1976.) £4.75.

PLANT physiological studies have contributed, and continue to contribute a great deal to plant ecology, and many universities and colleges now teach undergraduate courses in ecophysiology. Many such courses have arisen from a desire to present plant physiology in its true evolutionary and environmental context. It is natural, therefore, that publishers have been anxious to provide suitable texts for such courses and the book reviewed here follows others by Etherington (*Environment and Plant Ecology*, Wiley, 1974) and Larcher (*Physiological Plant Ecology*, Springer, 1975).

Bannister covers the expected ground—energy balance, responses to light and temperature, soils, mineral and water relations and competitive interactions. The approach is concise and basic. He starts from first principles and relies mainly on verbal rather than numerical explanation of the principles discussed. This makes the text readable; also, it is rich in pertinent illustrations, largely drawn from British examples.

The attempt to portray such a wide and complex subject at an elementary level has necessitated a considerable degree of selection and pruning, and some topics are dealt with very briefly and may, consequently, be oversimplified. As a general criticism, I feel that too little information has been provided on relevant metabolic processes, which leaves one rather dissatisfied with some explanations. For example, an interesting collation of data concerning the tolerance of extreme temperatures by plant species stops short of explaining their metabolic consequences or the biochemical adaptations involved. At times the avoidance of biochemistry even obscures the text, as in the case of the C-4 photosynthetic system and the explanation of photorespiration. The same, however, applies to Etherington's book on this point.

Bannister's book stands out from the crowd by virtue of its rich use of examples of field problems which have been illuminated by physiological work. One can point students to it with the confidence that it will simulate their interest.

P. D. Moore

P. D. Moore is Lecturer in the Department of Plant Sciences at King's College, University of London, UK.

newly on the market

These descriptions are prepared by the staff of *Nature* on the basis of material provided by manufacturers. There is a Reader Enquiry Form facing page 28.

25 MHz oscilloscope. Pye Unicam. A 25 MHz oscilloscope with full digital measuring facilities found normally only on higher frequency models, the Philips PM 3214 has all the features of a general purposes oscilloscope with high 2 mV sensitivity over the full bandwidth. Digital measurements are facilitated by such features as a delayed timebase with fully calibrated delay and an alternate timebase facility which allows display of both main and delayed timebase signals at the same time for direct and easy reference. The wide range of power supply possibilities allows the oscilloscope to operate in the laboratory or the field. Mains supplies of 110, 127, 220 and 240 V a.c. $\pm 10\%$ at 46–440 Hz, 21–27 V d.c. supplies or an internal 24 V battery option are the alternatives. Power consumption of the oscilloscope is 30 W.

Circle No. 42 on Reader Enquiry Form

Fluorescence detector. Perkin-Elmer Ltd. A new fluorescence detector for liquid chromatography, LC-1000, has been designed with emphasis on high sensitivity, selectivity and ease of operation. The LC-1000 uses a xenon source providing a continuum over the UV/visible region. The square section quartz flow cell of 20 μ l volume is designed to provide maximum sensitivity, minimum scatter and no detectable loss of peak resolution while still being simple to dismantle for cleaning. Model LC-1000 uses a continuous interference filter as an emission monochromator operating over the region 390–750 nm, and the ability to scan an emission spectrum of a peak in a chromatogram can provide peak identification—a unique advantage. For wavelengths below 390 nm the fluorescence can be monitored by any fixed interference filter.

Circle No. 43 on Reader Enquiry Form

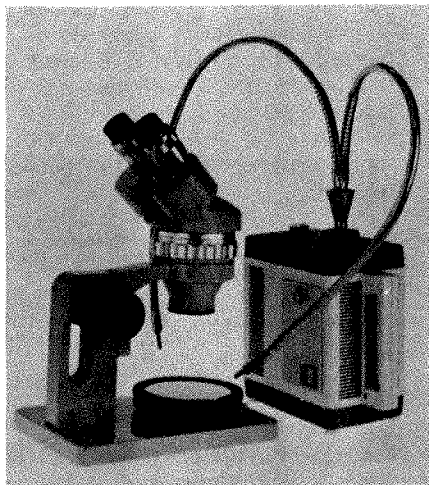
Photomultiplier power supply. EMI. The Type PM28A has the following characteristics from the electrical specification: output voltages from 100 to 2,800 V, ripple better than 2 mV peak-to-peak, temperature coefficient less than 50 p.p.m. per $^{\circ}$ C and load regulation 10 p.p.m. from a no load to full load (5 mA) change. An additional

feature is that the unit can be reset to within 0.2 V throughout the voltage range so that photomultiplier tube gains can be reproduced accurately. The unit is capable of positive or negative output, with options for remote programming and kits are available to mount units either singly or two abreast into a 19-inch rack.

Circle No. 44 on Reader Enquiry Form

Multiple sample harvesters. Ilacon Ltd. Designed for use in lymphocyte transformation assays, and a wide range of other isotope assays carried out in microplates, these harvesters introduce two unique design features which ensure trouble free and efficient operation. The basic model uses a patent disk cutter system to cut and hold the glass fibre filter paper, and a wash trough which allows complete versatility in washing without the need for switches or valves. Alternative models allow either supernatant collection or a disk clamping system for harvesting on to intact sheets of filter paper.

Circle No. 45 on Reader Enquiry Form



Will Z-33 zoom stereomicroscope.

Will Z-33 zoom stereomicroscope. Will-Strübin. The Will-Strübin stereo zoom microscope has a magnification range from $\times 2.7$ to $\times 180$. The eyepieces give extra wide field vision, and the eyepiece sleeves are adjustable and inclined 45° which guarantees comfortable vision in the normal sitting position. Various accessories are available including transmitted light universal stands, camera attachments, eyepieces $\times 5$, $\times 10$, $\times 15$, and $\times 20$, also auxiliary lenses and fibre optics.

Circle No. 46 on Reader Enquiry Form

Multipoint thermal printing recorder. International Instruments Ltd. The Speed Servo II combines a linear servo motor with a simple solid state semiconductor printing device that ensures positive multipoint channel identification. The printhead thermally brands the three basic types of readout: the channel number and a dot, all dots, or mixed dots and numbers as programmed. Models with up to 24 independent channels are available and the print rate can be as fast as one second per point full scale. The channel identification numbering system automatically prevents confusion when input values cause the traces to overlap, especially when all channels are in use, saving time in chart reading and analysis.

Circle No. 47 on Reader Enquiry Form

Liquid chromatographs. Perkin-Elmer. Series 2/1 is a single module providing both solvent delivery and sample injection and features high pressure capability, rapid solvent changeover, unlimited solvent supply, precise and steady flow, and a wide flow range adjustable from 0.1 ml min $^{-1}$ to 29 ml min $^{-1}$ for analytical and preparative separations. Series 2/2 combines the operating features of two Series 2/1 pumps and a sample injection system. The instrument provides linear solvent and flow programming capability from 0.1 ml min $^{-1}$ to 30 ml min $^{-1}$. Series 2/2 also offers direct mixing of selected solvent compositions, preparative flow rates, and allows independent solvent changeover.

Circle No. 48 on Reader Enquiry Form

Bench-top chemostat. New Brunswick Scientific Co., Inc. This accurately defines growth parameters such as agitation, aeration, temperature, nutrient addition and harvesting of cells grown in pure culture. The BioFlo can also be equipped with miniature probes and controllers for dissolved oxygen and pH regulation. With a working volume of only 350 ml, it is possible to sustain cell growth for a minimum of 38 h without replenishing the medium. The BioFlo can be operated at temperatures from below ambient to 60 $^{\circ}$ C. A magnetically coupled agitator produces a homogeneous dispersion of culture medium under aseptic conditions at speeds from 200 to 1,000 r.p.m.

Circle No. 49 on Reader Enquiry Form

announcements

Meetings

- 18–19 May, **Evolutionary Systematics of Bivalve Molluscs**, London (P. B. Carter, The Royal Society, 6 Carlton House Terrace, London SW1).
- 20 May, **Digital Satellite Communications**, London (The Institution of Electrical Engineers, Savoy Place, London WC2).
- 25 May, **World Energy Prospects to the Year 2000**, London (P. B. Carter, The Royal Society, 6 Carlton House Terrace, London SW1).
- 30 May–3 June, **American Geophysical Union Annual Meeting**, Washington, D.C. (AGU, Sheraton Park, Woodley Road, N.W., Washington, D.C. 20008).
- 1–2 June, **Essential Fatty Acids and Disease**, London (Royal Society of Medicine, 1 Wimpole Street, London W1).
- 4–6 July, **Rare Earths and Actinides**, Durham (The Institute of Physics, 47 Belgrave Square, London SW1).
- 10–22 July, **Sensory Ecology—Nato Advanced Study Institute**, Quebec (M. A. Ali, Biologie, Université de Montréal, Case Postale 6128, Montréal).
- 11–13 July, **Physical Chemistry and Hydrodynamics**, Oxford (D. Brian Spalding, Mechanical Engineering, Imperial College, Exhibition Road, London SW7).
- 13–15 July, **Processing, Structure, Properties and Performance of Polymers**, Nottingham (The Institute of Physics, 47 Belgrave Square, London SW1).
- 13–15 July, **3rd International Workshop on Phosphate and other Minerals**, Madrid (A. Rapado, Unidad, Metalibrica, Fundacion Jimenez Diaz, Av. Reyes Catolicos, 2, Madrid-3, Spain).
- 18–29 July, **Energy for Energy Decision Makers**, Cambridge, MA (Room E19-356, MIT, Cambridge, MA 02139).
- 18–23 July, **XXVII International Congress of Physiological Sciences**, Paris (J. Scherrer, U.E.R. Pitie-Salpetrière, Cedex 1300, F-75300, Paris).
- 24–30 July, **Optimal Development and Management of Groundwater**, Birmingham (J. W. Lloyd, International Association of Hydrogeologists, Geological Sciences, University of Birmingham, UK).
- 24–30 July, **40th Annual Meeting of the Meteoritical Society**, Cambridge (E. Scott, Mineralogy and Petrology, Downing Place, Cambridge, UK).

25–29 July, **6th AIRAPT International High Pressure Conference—Applications and Techniques**, Boulder (K. D. Timmerhaus, Engineering Research Centre AD-1-25, University of Colorado, Boulder, Colorado 80309).

25–28 July, **Synthesis in Organic Chemistry**, Oxford (J. F. Gibson, The Chemical Society, Burlington House, London W1).

26–29 July, **Electron Transport/Molecular Solids**, Leeds (G. J. Morgan, Physics, University of Leeds, UK).

2–5 August, **Cryogenics—A Century of Progress, A Challenge for the Future**, Boulder (K. D. Timmerhaus, University of Colorado, Boulder, Colorado 80302).

7–13 August, **8th International Conference on General Relativity and Gravitation**, Waterloo, Ontario (M. A. McKiernan, Mathematics, University of Waterloo, Ontario, Canada).

21–27 August, **5th International Conference on Birth Defects**, Montreal (International Birth Defects Congress Ltd, 1275 Mamaronek Ave, White Plains, New York 10605).

22–26 August, **International Symposium on Microbial Ecology**, Dunedin, New Zealand (The Royal Society of New Zealand, PO Box 12249, Wellington, N.Z.).

22–24 August, **Symposium on Scientific Instruments: Their Social and Economic Settings**, Greenwich (Navigation and Astronomy, National Maritime Museum, Greenwich, London SE10).

29 August–2 September, **48th ANZAAS Congress**, Melbourne (Secretariat, 48th ANZAAS Congress, PO Box 29, Parkville, Victoria, Australia 3052).

29 August–1 September, **6th International Symposium on Drugs Affecting Lipid Metabolism**, Philadelphia (W. L. Holmes, Lankenau Hospital, Philadelphia, Pennsylvania 19151).

30 August–1 September, **Dopamine**, Southampton (P. J. Roberts, Physiology and Miochemistry, University of Southampton, UK).

30 August–2 September, **Biological Activity and Chemical Structure**, Noordwijkerhout (Netherlands) (Secretary, c/o Merck Sharpe and Dohme B.V. Waarderweg 39, PO Box 581, Haarlem, Netherlands).

30 August–3 September, **4th European Crystallographic Meeting**, Oxford (C. K. Prout, Chemical Crystallography Laboratory, 9 Parks Road, Oxford).

31 August–2 September, **1st New Zealand Astronomy and Astrophysics Conference**, Christchurch (Secretary, Physics, University of Canterbury, Private Bag, Christchurch, N.Z.).

4–9 September, **13th International Symposium on Free Radicals**, Lyndhurst, Hampshire (A. Carrington, Chemistry, The University, Southampton, UK).

4–9 September, **4th International Congress on Photosynthesis**, Reading (J. Coombs, c/o Tate and Lyle Ltd, P. Box 68, Reading, UK).

5–8 September, **New Processes of Waste Water Treatment and Recovery**, London (D. H. Sharp, Society of Chemical Industry, 14 Belgrave Square, London SW1).

5–10 September, **4th International Round Table on Sintering**, Dubrovnik (ETAN, PO Box 356, 11001 Beograd, Yugoslavia).

Person to Person

Exchange 4 bedroom house just south of Cambridge, England for a house conveniently situated for Stanford University, California for a period of one year beginning August/September. Please contact Dr Peter Twentyman, MRC Clinical Oncology Unit, Hills Road, Cambridge.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

8–19 August, **Physics of Quantum Electronics**, Telluride, Colorado (S. F. Jacobs, Rt. 2, Box 732D, Tucson, Arizona 85715).

10–19 August, **XVth International Congress of the History of Science**, Edinburgh (E. G. Forbes, The Royal Society of Edinburgh, 22 George Street, Edinburgh, UK).

11–13 August, **Energy Conservation in Crop Production**, Palmerston North, New Zealand (Agronomy, Massey Department, Palmerston North, N.Z.).

15–18 August, **5th International Conference on Chemical Thermodynamics**, Ronneby, Sweden (Chemical Centre, Lund University, P.O.B. 740, S-220 07 Lund, Sweden).

nature

12 May 1977

A modest proposal to the Department of Education and Science

THERE is by no means enough lobbying of government on behalf of science. That was the challenge thrown out last week by Mrs Shirley Williams, Britain's Secretary of State for Education and Science, at a gathering of the Association of British Science Writers. The Arts, for which she is also responsible, lobby regularly and aggressively; science's voice is rarely heard. This will come as a surprise to many scientists who have assumed that lobbying would be unwelcome and counter-productive. But the challenge is not just to scientists; it is to Mrs Williams herself to find ways of opening up the London machinery to the community at large so that access to decision-makers becomes easier to achieve.

That British scientists don't do enough to express their opinions is undeniable. Certainly when an issue like the payment of CERN's subscription comes up there is a great flurry of activity, but for the most part the British scientist is totally silent—content, presumably, with zero growth rates and a secure job. Maybe the assumption is that the media will look after all questions concerned with policy and will spot all issues which ought to be raised; and then, when there is nothing in *Nature*, *New Scientist* or the *Times Higher Education Supplement*, it is assumed that there is really nothing to worry about. This would be a dangerous assumption; the media cannot hunt out what it knows nothing about, and certainly in the past few years the number of individual scientists who have approached us with a request that we should look into something or pursue a particular campaign is pathetically small.

Or maybe it is widely believed that worthy bodies like the Advisory Board for the Research Councils or the individual research councils themselves adequately look after the interests of science. The trouble with that assumption is that these bodies already have a fairly clear and well-charted relationship with the Department of Education and Science, and this allows relatively limited scope for anything resembling lobbying, which would, in any case, have to be done by chairmen themselves. What,

presumably, Mrs Williams is looking for is a regular flow of opinion of diverse sorts, not just from research council heads, on how support for science should develop.

It would be too easy, however, to lay the blame for the lack of lobbying entirely on apathy in the country. That apathy has very largely been fostered by the way in which British society divides its institutions so scrupulously. There is Parliament. There is the civil service. There is the law. There is industry and commerce. There is the military. There is the academic world. And there is the press. All too rarely do people find it easy to cross these frontiers and live happily in two or more camps either concurrently or consecutively, and as a result public life suffers through narrowed horizons.

Obviously there is no simple remedy to this. Over-secure career prospects and innate beliefs that certain ways of earning a living are superior to others are not going to be done away with overnight. But there is some positive action Mrs Williams could take to get to hear the scientist's voice; she could create a post for a distinguished scientist who with a small staff could act as the department's ears and eyes into the scientific community—and not just academics. Maybe such a person would be called Chief Scientist, but his or her function would be rather different from those of chief scientists in other ministries. In many ways the job of being a link with practising scientists used to be performed by the government's chief scientific adviser, but now that this position has been allowed to lapse there is no one to whom scientists can turn in a personal capacity. The department's chief scientist ought to fulfil that role, but in doing so ought to be highly mobile. The attractions of life in London close to the corridors of power are obvious, but the right person would not be bound to an office but would be regularly on the road, thereby not just hearing what institutions and professional bodies have to say, but more importantly seeking out the opinions of individuals. □

Today's risks: thinking the unthinkable

Reg Farmer, Safety Adviser to the UK Atomic Energy Authority and Visiting Professor at Imperial College of Science and Technology, offers his perspective on risk in modern society

THERE is growing concern over the safety of our industrial and social activities. People ask, "Is it safe?" For many years we have been told about the risks from radiation; now we hear of the potential hazards to people or the environment from mercury, lead, asbestos, aerosols, and recently from saccharin.

There have been various attempts to draw a dividing line between 'safe' and 'unsafe' or even to establish absolute safety—a state of no risk. In the early days of atomic energy in the UK, there was an attempt to find a design of reactors which was inherently safe—any move to danger would be self-cancelling. More recently some people claim that if any risk can be identified, it must be eliminated. Neither of these approaches has proved to be achievable in practice.

In the USA it was first implied that reactors would be safe if contained, and there was a proposal to design a 'zero release' containment, so as to appear to give absolute safety. Later there grew a recognition of some risk—in the USA a three-man hearing was required to satisfy itself that the proposed nuclear plant created no undue risk. 'Undue' did not mean no risk, but it introduced the concept of reasonable. No specific directive was given as to that which might be judged reasonable other than through the circular statement:

To understand 'undue risk' in this context it is necessary to understand 'reasonable assurance' and AEC has defined this as meaning 'reasonable probability, establishing under the particular circumstances of the case in good faith and in the exercise of sound discretion and expert judgment.'

A not dissimilar situation arises in relation to the use of some chemicals, fertilisers, insecticides, food additives and so on. Generally their use should be controlled by the best possible means. In some cases and in some countries absolute criteria apply—as in the US Food and Drug Administration requirement that any foodstuff shown to produce cancer shall be banned. It is not then a question of what is reasonable, but a pursuit of no risk. This absolute criterion is not in keeping with the acceptance of risk at work, at play, or risk through the continued low level contamination of our environment.

It would be convenient if the answer to the question, "Is it safe?" could be yes or no. For thirty years, throughout the development of atomic energy, there have been many and varied attempts to derive criteria which, if satisfied, would imply safety or adequate safety and, if not satisfied, would imply some risk. For some years it was claimed that pressure vessels could not fail catastrophically if they were maintained at some appropriate temperature (nil-ductility temperature + 60 °F). There have been many attempts to find an all-embracing solution, by proposing an upper limit accident which the system should be designed to tolerate safely, to circumscribe all lesser accidents—such as the assumption that the largest pipe could fail or that, in gas-cooled reactors, the coolant flow might cease instantaneously.

These early accident studies followed a pattern, now called deterministic; this emerged when it was recognised that, in general, no system is inherently safe. It was at one time suggested that we should take account of two co-incidental faults, but not three. This approach has been

revised in various ways, such as a requirement that there should be two or three 'boundaries' between fission products and man. These requirements are largely illusory and do not define what risk level is being accepted.

I would like now to consider some other aspects of the current risk scene. It would appear that some hazards are thought to be more unacceptable than others, such as the results of thalidomide, the effect of dioxin or of radiation. It is easy to understand the horror of continued evidence of mistake or misfortune, as presented by malformed children. It is not easy to identify or justify the fear of exposure to radiation as against exposure to toxic chemicals.

Radiation and radioactive substances have been with us always; some say the new situation arises because of the bomb; others that it arises from the fear of cancer, as may be evidenced by the USA ruling on the prohibition of any potentially carcinogenic substances in food. If I were given the choice of death today by accident, or death in 10–20 years by cancer, which would I choose? There is some chance—about 1 in 5—of dying by cancer from so-called natural causes. Alternatively, and indeed more likely in an accident, how would I rate a chance of 1 in 10 to 1 in 100 of immediate death or death within a short time through explosions or toxic gases, as against the same risk of death in 10 to 20 years? I would choose to live on.

It is believed that radiation presents new risks, but radiation has always been with us, and carcinogenic or genetic damage does not arise solely from radiation, as reported by Sir Edward Pochin¹. Apart from 'a different sort of hazard' from radiation, it was thought that accidents to nuclear sites might cause greater harm than hitherto accepted in modern society. In my view this is not so, and at this point I approach the nub of this present discourse, that is, how best or in what way to describe the results of a hypothetical accident to an industrial site?

In my early days in nuclear energy, it was customary to talk about 'maximum credible accidents' and particularly so in the USA, but in general so to circumscribe the conditions of the hypothetical accident as to make the 'mca' acceptable, so that the installation might be licensed. However, some writers describe accidents beyond those currently reviewed in the traditional 'mca' approach, as in the early document, WASH-740, describing in 1957 the possibility of 3,400 deaths and more than 10,000 injuries. Somewhat earlier, in 1955, at Geneva official papers described hazards from radioactive substances as one million to one thousand million times more dangerous than chemical poisons, whereas it is now generally accepted that plutonium is only about ten times more toxic than chlorine. This violent see-saw, from the trivial to the disastrous, has dogged the development of atomic energy. We need to come to terms with this situation. Perhaps both ends of the scale are possible and both represent half-truths, but we may penalise the further development not only of energy resources but also of our industrial and technological development unless we have a better understanding of the nature of hazards and their associated risk rates.

An accident in any major industry may lead to financial loss, the shutdown of plant; it may lead to no harm, or the same accident might cause many casualties. There is some chance of an accident occurring in most industrial installations or in transport. The consequences will depend upon many things: the quantity of toxic or explosive material available; the quantity released in the accident; the rate of release; whether the release occurs in seconds, minutes or hours; the surrounding population distribution; the conditions of wind and weather; topography; the effectiveness of remedial procedures on and off site.

Some of these are reasonably independent or capable of prior assessment. In many cases the accident is not caused by wind or weather, but the consequences may be greatly influenced by them. In some cases accidents are caused and may be predicted from unusual weather patterns—flood, high wind, low temperature and so on. In general, there is no clearly defined maximum; it is possible to derive more and more casualties by presupposing more unusual conditions—the limit or maximum remains vague and unapproachable. Yet this idea of 'worst' or 'maximum' cannot be too lightly discarded. In financial matters it is usual to have a defined limit of liability; by the same token it would be desirable to have an upper limit of hazard which may then be accepted or rejected.

Unfortunately this route is not yet open to us, and is certainly one not easy to follow. In recent history it has been the practice to describe the worst accident to reactors or to some industrial plants in terms of some framework of credibility, generally so framed as to make the consequences of the accident relatively small and not too difficult to accept.

There is another route—an alternative to the synthetic analysis of a hypothetical accident. This route is to list and survey the accidents which have occurred in certain fields—ground or air transport, petrochemicals, the manufacture of chlorine and so on—and to show that from some dozen or so accidents only a few people are killed. It is then fair to extrapolate to the future if the analysis takes account of any change in toxicity and quantity of material at risk and any improvement in the means for their control. Indeed, estimates prepared in this way give a good indication of the 'likely' results of an accident.

To return to 'the worst'. Until recently it might 'reasonably' have been said that the worst aircraft accident might kill 350 people. There was always some risk that two might collide and this has happened. It could be worse. USA studies reported in evidence to a Senate Committee⁷ have given a probability of an aircraft crash on Hollywood Park grandstand whilst occupied by a large crowd as about 10^{-3} per year and the chance of killing 100–10,000 as being in the range 10^{-5} – 10^{-7} per year. In this simple example, it could be that of two groups discussing risks in air transport, one could believe that 350 casualties represented the 'worst' whereas the other could fear the risk of 10,000 casualties.

An even greater range could apply to some industrial accidents. The accidental release of toxic or explosive gases has caused 10–20 deaths on several occasions. A retrospective analysis shows that in some cases, had the wind been blowing in another direction, the result might have been ten times worse. Furthermore, had the weather pattern been less favourable, the casualties would have been greater. It is known that under inversion conditions, with low wind speed, the downwind air concentration for a given release of material is up to ten times higher at a given distance than for average weather conditions, and that in the UK, inversion occurs about 15% of the time—a small chance, but still significant.

There has been considerable effort employed in assessing the chance of major hazards from man-made, or natural disasters, as from explosion, fire, earthquake, or even meteorite impact. These studies require a combination of two factors: extrapolation from past history weighted according to the judgment of the assessor; and the use of currently available data on probability of wind direction, weather category, population distribution.

Many small accidents have occurred, and will continue to occur, which kill from 1–10 people. There is some small chance that a combination of circumstances might cause the death of over 100 people. Rasmussen² and Okrent³ have explored a range of possible hazards in the USA and, for each of various categories of fire, explosion and so on, have given their prediction as follows:

Chance of killing more than 100 people:	10^{-1} – 10^{-2} per year
Chance of killing more than 1,000 people:	10^{-2} – 10^{-4} per year
For earthquakes, they estimate:	
Chance of killing more than 10,000 people:	$\sim 10^{-2}$ per year
And for dam failures:	
Chance of killing more than 10,000 people:	$\sim 10^{-3}$ per year
Their assessment for overall risk from all recognised sources gives:	
Chance of killing more than 1,000 people:	10^{-1} per year
Chance of killing more than 10,000 people:	in the range 10^{-2} – 10^{-3} per year

The situation in the USA is different from that in the UK. Natural hazards from tornadoes, dams and earthquakes are greater in the USA. On the other hand the UK population density is roughly 10 times that of the USA. My own view is that the comparable risk in the UK lies within a factor of 10 of the USA—perhaps a factor of 10 lower. But on this matter we should not be too complacent.

These figures may be frightening, and it would be terrible if 1,000 to 10,000 people were killed; it is unlikely, but it could happen and is experienced in other countries from earthquakes and floods. Even so, a single event of this type would barely change the overall accidental death rate in the USA or Europe. The overall accidental death rate is about 1 in 2,500 per year, although this is age dependent. Most of us have a 2% chance of accidental death in 50 years. In a country of 50 million people, this means that more than 20,000 die from theoretically avoidable accidental death (including suicides) each year.

In giving guidance on some new activity, there is need to take account of both current risk levels and public reaction but, in so doing, it is important that the public should be informed and that they should recognise the need for wise and balanced judgment in situations where absolute safety can no longer exist. The public seem to expect the experts to seek ways of reducing risk and if ways are discovered which promise clear benefit at not too great cost, they expect the remedy to be applied.

Additionally, I believe that there are pressures to reduce risk irrespective of the risk rate, or in the absence of specific knowledge of the risk rate. There will be pressure to reduce risks in atomic energy, in aircraft flight and ground transport, and in many industries, such as mining, fishing, chemicals, petrochemicals, steel, oil exploration; and also pressure to spend more money in medical research and health facilities as a means of reducing risk of suffering or death.

How best to resolve these pressures on money and skill? How best to decide whether to reduce risks still lower in places where they are already low, as against the need to reduce the frequent and relatively random causes of common accidents? There is no easy answer.

I believe that progress should be made in two directions: to improve communication between people on this matter of risk of hazards to life and the environment; and to continue to improve the techniques for the assessment of the risk of future harm to man and environment. A considerable effort is being deployed in both directions in the atomic energy field and is now being extended more widely in our industrial activities. □

¹ Pochin, E. E. *Estimated Population Exposure from Nuclear Power Production and Other Radiation Sources*, NEA/OECD, 1976.

² WASH-1400, *Reactor Safety Study—An Assessment of Accident Risks in US Commercial Nuclear Power Plants*, USNRC, October 1975.

³ Okrent, D. Investigation of Charges relating to Nuclear Reactor Safety, Hearings before the Joint Committee on Atomic Energy, Congress of the United States, 94 Congress, Second Session, 2, Appendixes 12–19.

Zoo story

Alastair Hay looks at the London Zoo, now experiencing some changes at the top

AT its Annual General Meeting this week, the Zoological Society of London (ZSL) was due to witness a major change in the composition of the Council, its governing body. Both the President, Prince Philip, and the Secretary, Lord Zuckerman, were resigning their offices, with Prince Philip leaving the Council altogether. The Council nominated Lord Zuckerman as President and Dr Ronald Hedley, the Director of the British Museum (Natural History), for the Secretaryship. The Secretary of the ZSL is the executive officer of the Council; when it was a salaried post in the years 1847–1943 it was one of the most sought-after positions by zoologists in Britain.

Three years after its inception in 1826—under the auspices of Sir Stamford Raffles (the first President) and the then President of the Royal Society Sir Humphrey Davy—the ZSL was incorporated by Royal Charter with the specific objects of “ensuring the advancement of zoology and animal physiology and the introduction of new and curious subjects of the animal kingdom”. Animal physiology at this time was concerned primarily with the optimal conditions for animal breeding. Thus, whereas Raffles was more interested in the scientific potential of the Zoo, Davy—in Raffles’ view—was more concerned with its “practical and immediate utility to the country gentlemen”.

Davy’s ideas notwithstanding, his support enabled the ZSL to develop rapidly into the centre for zoological discussion in Britain. In previous years the Linnean Society had been the hub of these discussions, although it was principally concerned with botany. There is some irony in the fact that the foundation of the ZSL was a reaction against this bias; the Linnean’s own development, in 1788 to cultivate “the science of natural history in all its branches”, was itself a reaction to the Royal Society’s own preoccupation with the physical sciences.

During its first hundred years the ZSL’s publications were mainly concerned with systematics, comparative anatomy, taxonomy and phylogeny. In this respect they very much reflected the interest of the Secretaries of the Society, men such as Philip Lutley Sclater (1859–1902) and Peter Chalmers Mitchell (1902–1935). But well before Sclater retired, the biological sciences were themselves undergoing rapid change. Zoology and animal phy-

siology were now sciences which required animal experimentation and a knowledge of genetics, biochemistry and endocrinology. The Establishment at the Zoo seemed to be unaware of these developments and consequently younger zoologists were looking elsewhere. The Society was losing the scientific support on which its integrity and very survival depended.

Huxley’s problems

Julian Huxley was the first of the experimental zoologists to be appointed Secretary. Although he attempted to change the Society’s approach to zoology he was, in the main, unsuccessful. By 1942 Huxley and the council were openly opposed to one another. Huxley resigned in 1943, an action which was regarded by many zoologists as a serious defeat for progressive scientists. For the next twelve years few changes were possible in the Society. The situation for the duration of the war and the first decade of post-war Britain required Huxley’s successors, Sheffield Neave and Lord Chaplin, to devote all available resources to restoration following bomb-damage and to the replenishment of the animal collections. Lord Zuckerman became Secretary in 1955 with the ZSL in a very poor state.

The ZSL received confirmation of its status as an educational charity in 1930. Lord Zuckerman believes implicitly in the importance of the Zoo as an educational institution and in the need for the Society to adhere to the terms of its Charter and his determination to institute changes along these lines and to bring it back into the mainstream of zoology involved him in a major clash with Fellows of the Society in 1957. At the Annual General Meeting that year he proposed, on behalf of the Council, an increase in Fellows’ subscriptions (unchanged for over 100 years), the creation of a new class of Scientific Fellow, and the extension of public visiting hours on Sundays.

The changes were essential if the Society was to preserve its charitable status and to increase its revenue. For many of the Fellows, however, these proposals represented a considerable challenge to their privileged status. As a result the 1957 meeting was probably one of the most lively in the Society’s history. The Council’s proposals were finally ratified, but not before the dispute was dragged into the High Court and finally settled in



Zoo guests

the Court of Appeal. The proposals and court ruling were incorporated in a new Royal Charter in 1963.

Ivor Montagu, one of the ZSL's Vice-Presidents and a regular visitor to the Zoo for nearly seventy years, believes that the 1957 proposals were absolutely essential for the Zoo's survival. In his view the strength of the institution lies in its Charter but the composition of the Council has sometimes made it vulnerable. He says it was set up in a way which grew alien to the world around it: the Zoo in its early days was very much a preserve of the gentry; a walk in the Zoological Gardens on Sundays by the Fellows and their friends was very much 'the thing to do'. The First World War altered the social climate and this, he says, forced the Fellows to relinquish some of their privileges. By pushing the 1957 proposals through, he argues, Lord Zuckerman "rescued the Zoo and brought it into the modern world." For many years London Zoo had indulged in the policy of 'stamp-collecting'—it had to have at least one specimen of each species. But with greater concern about conservation and the alteration in public attitudes that policy changed. The emphasis is now placed on maintaining fewer varieties of animals and these in viable breeding colonies.

Zoo activities

The ZSL is an institution which maintains the national collection of animals. It owns one of the most comprehensive zoological libraries in the world and is responsible for an extensive educational scheme which catered for 53,000 pupils in 1976. The Society publishes more scientific journals than even the Royal Society and

in addition it runs two research institutes of world renown. These activities, undertaken in addition to the normal display of animals, are financed in the main by gate proceeds. The ZSL's 1976 Annual Report states that with a turnover of some £2.5 million and a combined attendance of close on 2½ million at Regent's Park and Whipsnade, the Society had a year-on surplus of £179,000. But this sum, unfortunately, will have to be used to pay off the deficits accumulated in 1974 and 1975 when the effects of inflation and recession were sorely felt.

The additional activities undertaken by the ZSL are an obvious strain on the Society's revenue. Both in Britain and abroad many other institutions with far fewer responsibilities are in receipt of regular government subventions. London Zoo is not so endowed. The obvious question is whether it should be. Lord Zuckerman is circumspect with his answer. All he says is that the ZSL is as worthy of support as many of the institutions in regular receipt of public funds.

An intense debate is being conducted at the moment by the staff within zoos to decide their real purpose—the educational function of zoos, their role in conservation of endangered animal species, the nature of scientific research to be carried out and so on. The 1976 Report acknowledges part of that discussion. With its facilities the Society is well placed to become "an international centre for the scientific study of the problems of conservation." The Council proposes to combine the two research institutes, the animal hospital and pathology laboratory into an integrated 'Institute of Zoology'. The scientific expertise and support of the

new institute in reproductive physiology, biochemistry, immunology, radiology, parasitology and genetics "can put real knowledge behind attempts to breed stocks of rare animals". This is to become one of the institute's main projects.

Finance for conservation research, however, is very difficult to find. Many zoologists feel that at least some of the money raised for wildlife preservation generally could be used more profitably for the scientific research needed to back up the conservation programme. One organisation concerned with conservation is the World Wildlife Fund. Its Vice-President and Founder Chairman, Sir Peter Scott, feels that conservation is important in the context of zoos. He says zoos are especially important in two respects: for the captive breeding of species threatened by extinction, and for education. Zoos have a great responsibility to educate the young, he argues, and the ZSL has done well in both areas; although the Fund did not at the moment finance any conservation research at London Zoo, if a good case was made they "would consider supporting it".

The ZSL's total income in 1976 was about £12,000. Some 16% of this was spent on scientific activities. Today the proportion of income spent on research, publications and education stands at the respectable level of 18%.

Hedley's task

Ronald Hedley, the man succeeding Lord Zuckermann as Secretary of the ZSL, is a biologist by training with a background in chemistry, zoology, geology and physiology. His experience at the Natural History Museum should equip him well to deal with the public and with the scientific and educational functions of the Zoo; last week he said he was looking forward to the prospect of working with new colleagues there. But he is not approaching his new job with any preconceived ideas. Believing in the need to sample opinion, of which there will be no shortage at the Zoo, he intends to have a period of consultation first.

The Council of the ZSL has the ultimate responsibility for ensuring that the Society is successful. Dr Hedley as a member of that Council now has the two-fold task of involvement in the running of the Zoo and the Natural History Museum, which is an essentially complementary institution. He is conscious that the goodwill and support of their staff is essential for their continuing success, and takes up his post when the Zoological Society of London is probably more confident about its future than at any time in the past fifty years. □

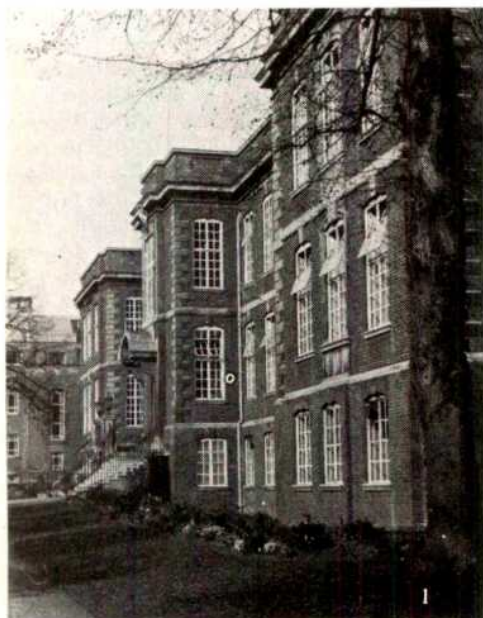
As of this week Lord Zuckerman will preside over all the Zoo's affairs and chair any of its meetings which he attends. Previously, as the Zoo's Secretary, he had a more demanding task, which is one obvious reason why he might retire from the post. But, though now 73, he will still be around to offer advice, just as he has done in another capacity, that of a government science adviser. This link has lasted for the best part of 40 years, and culminated in promotion to Chief Scientific Adviser from 1964 to 1971. He is still to be found in and around the corridors of power.

Interviewed last week, Lord Zuckerman responded firmly to the suggestion that with the explosion of information no single individual could possibly advise over the whole spectrum of science. What was wanted of a scientific adviser, he argued, was an understanding of the "political, national and international" implications of technical decisions taken by governments; the immense political implications of President Carter's decision not to proceed with the breeder reactor, for example. That a political sense was required was particularly true

when all government departments already had specialist technical committees to consider long term planning decisions. Zuckerman says that "the idea of appointing a scientist just because he is a Nobel Laureate who has unravelled the structure of some particularly heavy protein molecule is nonsense. The idea of having the best mathematician is nonsense".

It needs no mathematician to calculate that Britain's Research Councils are now having a comparatively lean time. Zuckerman acknowledges that in the 1950s and 1960s he was one of the individuals who convinced people that science should be supported in a much bigger way and that now people have the right to enquire after the results of that policy. With the golden age of expansion over, some scientists will draw little comfort from his belief that they will just have to accept the cuts as a fact of life in a country as "hard-up" as Britain. But he believes the current period of belt-tightening will not restrict "real creative thought", and that there will be no long-term repercussions for scientific output.

Fiftieth anniversary of the Dunn School of Pathology



- 1 The Sir William Dunn School of Pathology
- 2 Dr Jean Medawar, Dr Anne Beloff-Chain, Lady Florey, Sir Peter Medawar, Sir Ernst Chain, Professor A. D. Gardner
- 3 Dr George Mackaness
- 4 Professor Dorothy Hodgkin, Professor D. Whitteridge
- 5 Professor Henry Harris



WHEN the trustees of Sir William Dunn's bequest offered to support the founding of a new pathology building in Oxford they stipulated that it should be fronted by flower beds. On 20 April, the occasion of the 50th anniversary of the opening of the Sir William Dunn School of Pathology, only the reminiscences of the plentiful past members of the School were more colourful and varied than the flowers outside. The celebrations were launched, in a style unequalled by later speakers, by Emeritus Professor A. D. Gardner. An Oxford graduate of 1907, Gardner recalled how Sir William Dunn's money, earmarked primarily to aid the destitute and the orphaned, came to be used for funding medical research. The School's first director was Professor Georges Dreyer whose strength, said Gardner, lay in his thorough statistical approach to research which was centred on the development of tuberculosis vaccines. His weakness lay in his belief that he was infallible—a vice, Gardner emphasised, that was unique neither to the man nor the era.

After Dreyer, in 1935, came Professor Howard Florey who was remembered in his pre-war days by Sir Peter Medawar for being exhilaratingly energetic and exasperatingly anti-intellectual. The war-time saga of the isolation and first therapeutic trials of penicillin by Florey's team was narrated by Dr Norman Heatley who emphasised the contributions of novel hardware (bedpans, biscuit tins and Russian butter tins) and novel,

hard-wearing female technicians. In 1952 when Henry Harris arrived from Sydney, Florey's direct interests in antibiotics were being wound up in J. L. Gowans' DPhil thesis. Thereafter antibiotic research was carried out under E. P. Abraham who told the story of cephalosporins from their bizarre origins in a Sardinian sewage outlet to the present day.

Florey's interests, ruled as ever according to Professor Harris by the illnesses with which he or members of his family were afflicted, turned to atherosclerosis, gastric secretion and inflammation. A three-pronged attack on inflammation was mounted by Florey through George Mackaness, Gowans and Harris who were to investigate the roles of the macrophage, the lymphocyte and the polymorph, respectively. Only Harris gave up. His decision that the polymorph had been good for a DPhil but would not do for a career brought out the fiercest in Florey. Harris was threatened with deportation to Australia, and communication between the two became confined to letters. Fortunately Harris's colleagues, particularly Abraham, rallied round and persuaded Florey to back down. Henry Harris survived to take over Florey's chair in 1963. We look forward to the 75th anniversary of the Sir William Dunn School of Pathology when tales of Henry Harris can best be told, perhaps under the shade of the ginkgo tree ceremonially planted by his wife on this occasion.

Peter Newmark

USA

The costs of high technology

A report last week on X-ray scanners has raised questions about expensive technology in the US health care system. Colin Norman reports from Washington

In mid-1973, a sophisticated and very expensive piece of medical equipment was introduced into the United States from Britain. Since then it has found its way into hundreds of hospitals and doctors' offices, and the swiftness with which the new technology has been adopted has generated a major controversy. This has helped shed light on the reasons why hospital costs have risen eight times faster than the cost of living during the past 25 years, and why many people despair that the American health care system can ever be brought under sensible cost controls.

The equipment, known as a computed tomographic (CT) scanner, is a nifty machine which its promoters claim will revolutionise the diagnosis of many medical disorders. Developed by EMI Ltd, the CT scanner is essentially a combination of an X-ray machine and a computer, which provides cross-sectional images of internal body organs. Early models were limited to use on the head, but later and more sophisticated versions can provide images of the internal organs of any part of the body. The scanner's advantage over conventional X-ray machines lies in its ability to produce high quality images of soft tissue structures from numerous angles.

Few people would quarrel with the argument that CT scanners represent a major advance in medical X-ray technology, or that they may be useful diagnostic tools in many instances. The controversy centres on the fact that the technology has been so widely and swiftly adopted in the United States that massive investments have been made before the clinical usefulness of the machines has been well documented.

Although less than four years have elapsed since the first CT scanners were introduced into the United States, an estimated 760 machines have now been installed or are on order. Since each machine has a price tag of between \$300,000 and \$700,000 and costs about \$300,000 a year to operate, hundreds of millions of dollars are being sunk into the technology by American hospitals. A particularly striking indication of the overwhelming demand for the machines is the fact that some 40 orders were placed for EMI's new whole body scanner before the machine

was even commercially available. By contrast, in Britain, where CT scanners were originally developed, only a handful of machines are in operation and fewer than 40 have been ordered.

This overwhelming and seemingly unreserved adoption of CT scanners in the United States led the Health Research Group, an organisation linked with the consumer advocate Ralph Nader, to charge last year that "the purchase of these machines is a particularly striking example of the introduction of extremely expensive medical technology into the health care system before there is adequate evidence that patient benefits or dollar savings exceed costs". The group demanded that a national moratorium be placed on the purchase of CT scanners until the benefits had been better evaluated. And last week, the Institute of Medicine, part of the National Academy of Sciences, said in a carefully worded report that "the long-term effects of CT scanning on medical care and its costs are not yet discernible".

The Institute's report, perhaps the most influential study yet published on the use of CT scanners, is noteworthy as much for what it says about the general problems of controlling hospital costs as for its recommendations about this particular technology.

The report is quick to acknowledge that CT scanners may have many important uses, and it lists some of the areas in which the machines may offer advantages over conventional diagnostic tools. Nevertheless, the Institute notes that conventional techniques may be just as effective and much cheaper in many cases, and "considers it essential . . . that controls be placed on the location and use of and charges for CT services". It also recommends that medical insurance companies should not pay for some types of CT scan until there is better evidence of their efficacy.

The question arises as to why the American hospital system has rushed to embrace CT scanners so wholeheartedly. The answer lies in the organisation and funding of the health care system, and the swift adoption of CT scanners epitomises many of the problems inherent in that system.

To begin with, there is little political incentive to hold down the costs of medical care in the United States. The middle classes, where political power largely resides, are generally well protected by private health insurance, and the medical costs incurred by the poor and the aged are met by the federal government. As the Institute puts it in

its report, "The growing share of personal health care expenditures covered by third-party reimbursement has reduced the incentives to control use (of expensive technologies), because the physicians decision to use services is separated from the patient's immediate expenses". According to officials in the Department of Health, Education and Welfare, more than 90% of the \$55,700 million hospital costs incurred last year were met by private insurance payments or federal Medicare and Medicaid schemes.

The report cites other reasons for the soaring costs, such as "competition among hospitals for medical staff, prestige, or revenues", and it notes that there has been a recent general increase in the use of diagnostic tests stemming partly from fear of malpractice suits, which "encourages defensive medical practice". All of these factors, the Institute states, suggest that "the present organisation of medical care and methods of financing and regulating that care in the United States have encouraged investment in beds and equipment beyond a socially efficient level".

Some states are trying to get a grip on soaring hospital costs through so-called certificate of need laws which require institutions to obtain approval from planning authorities before purchasing expensive equipment. But these laws, for a variety of reasons, have failed to curb the proliferation of CT scanners. The Institute report notes, for example, that some 15% of the CT scanners installed so far are located in the offices of private physicians, which are exempt from the certificate of need requirements, and "some physicians appear to have deliberately circumvented the intent" of the laws. In one well-publicised case, for example, after a hospital was denied a CT scanner, physicians at the hospital formed a partnership, bought a machine and installed it in an office across the street.

A final incentive for hospitals to purchase CT scanners seems to be their potential for generating revenues. The Institutes report states, for example, that the average charge for a scan is about \$240, and an independent economic survey of CT scanners published last year indicated that the average machine is used for about 3,000 scans per year. In other words, about \$700,000 is generated by each machine per year, more than enough to offset the purchase price and operating costs over a very short period.

Given that profit potential, there is some incentive for hospitals to use the machine in cases where less costly, conventional diagnostic equipment would

USA

● Total spending on research and development in the United States is expected to reach nearly \$41,000 million this year, according to figures compiled by the National Science Foundation. If the estimates are correct, support for research and development would stay ahead of inflation for the second year running, though the combined two-year increase would not be sufficient to wipe out inflationary losses incurred during the early 1970s.

According to the NSF study, the federal government is expected to spend about \$21,800 million in 1977, a 10% increase over 1976, while industry is expected to spend about \$17,500 million and about \$1,500 million will come from universities, non-profit institutions and so on. In terms of constant dollars, the total is expected to be about 6% below the peak spending years of the late 1960s. Measured as a proportion of the gross national product, spending on research and development has declined steadily since 1964.

NSF has also published an estimate of the number of scientists and engineers employed in 1976, which suggests that after four years of growth, the scientific labour force now numbers 542,000. The total is about 20,000 below the 1969 level, however. Some 40,000 science and engineering jobs were lost during the 1969-1973 cutbacks, NSF reckons.

● While the nuclear industry in the United States was licking its wounds last month, following President Carter's decision to defer commercial reprocessing and to downgrade the breeder reactor programme, a report on nuclear power plant security drew some fresh blood. Published by the General Accounting Office (GAO), an investigatory agency of the Congress, the report concludes that "security systems at perhaps all power plants would not be able to

withstand sabotage attempts by threats that are now considered minimum by (the Nuclear Regulatory Commission)".

Based on inspections of security systems at six power plants, carried out by GAO investigators, the report is written in uncharacteristically blunt language, laying blame at the doors of both the nuclear industry and NRC. It states that though the quality of security forces seems to vary greatly from plant to plant, some deficiencies were encountered on each inspection. The faults included lack of training of security guards (as little as four hours at one plant), high turnover of security personnel (up to 48% a year), and poor security equipment. In particular, the report cites the following two horror stories:

We accompanied an NRC inspector to one power plant at night. The inspector asked the guard manning the guardhouse to aim a closed circuit television camera on a particular spot. The guard tried but was unable to work the system. The inspector opened a door which rang an alarm in the guard house. After waiting several minutes, the guardhouse was called to find out why no one responded to the alarm. A guard in the guardhouse answered that all of the available guards were too busy.

At yet another site, we asked a guard about the locations of certain critical systems of the plant, including the control room. He told us that the guard force knew nothing about the location of these systems because the guards were not allowed inside the power plant.

Although the NRC has recently published a set of new regulations designed to increase the effectiveness of security systems at nuclear plants, the regulations are not due to come into effect until August 1978. The GAO report says that although the new regulations are "on the right track", NRC should take steps immediately to ensure that operating plants are made more secure.

Colin Norman

serve just as well. To try to limit such potential abuses, the Institute recommends that medical insurance companies should establish an advisory committee to develop criteria for paying CT scan charges. It also recommends that requests for use of CT scans should be reviewed by a physician with responsibility to control access to the machine, who would determine whether the scan is appropriate.

The central question here is not whether CT scanners are useful, but what level of investment can be justified and how the machines should be allocated and managed. Those are questions that the American health

care system finds difficult to address, given its fragmented nature, the competition between hospitals for revenues and prestige and the third-party payment system. As Dr Harvey Fineberg, Director of the Graduate Program in Health Policy and Management at Harvard School of Public Health put it last week, "The fundamental assumption that we in this country have not yet made is that the pot of resources available for health care is limited . . . if you ask whether CT scanners are useful, the answer would be 'yes', but if you frame the question 'would they be a sound investment', you may come up with a different answer". □

WEST GERMANY

Energy plan published

Werner Gries reports from Bonn on the energy research programme recently approved by the West German government

THE main emphasis of the German federal government's DM 6,200 million energy research programme for 1977 to 1980, published at the end of last month, is on nuclear research, but the rational use of energy, progress in coal technology and the development of new energy sources are also prominent. Details of the programme, which marks the first time the state has intervened to bring together systematically both nuclear research and work on other energy resources, are as follows:

Nuclear energy will take some 75% of the state funds. Fast breeder and high temperature reactors remain the first priorities in the country's nuclear power programme, but research and development work is to continue in the fields of uranium enrichment, reprocessing and the disposal of radioactive waste. A state subsidy of about DM 1,200 million will be needed just to complete the prototype power stations now under construction to house the breeder and high temperature reactors. Reactor safety is a key aspect of the programme; R&D on nuclear-powered ships will be continued only on a fairly modest scale, receiving about DM20 million annually.

Rational energy use: Around DM100 million annually will go into research into technologies for rational energy use. Attention will be given to techniques of remote heat supply, reverse cycle heating systems, heat recovery processes and the use of waste heat from power stations.

Coal technologies: R&D in coal technology, one of the most important aspects of the non-nuclear side of the programme, will receive increasing amounts of funding, amounting to an average of DM140 million annually. Coal gasification, improvements to coal-fired power stations to reduce pollution, coal liquefaction and improved mining techniques will all receive support.

New energy sources: The government will support the development of new sources of energy to the tune of DM130 million a year on average, with the focus on nuclear fusion (about DM90 million annually) and on solar energy. In the government's view, solar energy is of interest in West Germany only as a means of heating build-

ings and providing hot water, and research is being concentrated in this area. Further work on nuclear fusion is dependent upon the site of JET; the Research and Technology Minister, Hans Matthöfer, expressed his hope for an early decision on this.

It is Herr Matthöfer's ministry which has much responsibility for R&D in West Germany. Its budget in 1977 of DM4 million million compares with total research expenditure in 1977 estimated at DM26 million million. Federal research programmes are designed for a period of about four years, classified by sectors and provided with a corresponding budget during the planning stage. The energy programme is one of ten programmes for

sectors that also include data processing, marine research and technology, raw materials, electronic components, space and aviation, and so on.

The outlines of the new energy programme were determined by the federal government following a cabinet resolution. The programme is not immediately binding for industry but outlines the frame of what the government considers essential. The country's primary energy consumption in 1975 broke down as follows:

Mineral oil	52.1%
Hard coal	19.1%
Soft coal	9.9%
Natural gas	14.0%
Nuclear energy	2.0%
Others	2.9%

The structure of energy consumption differs from the United States and other large countries. A breakdown of energy consumption by sectors in 1975 shows that industry consumed 35.9%, transport 19.7% and domestic 44.4%. In the government's opinion the largest savings have to be achieved in the domestic sector. Industrial energy consumption, related to national product, has actually stagnated for about ten years.

An output capacity of 33,000 MW from nuclear power stations in Germany was anticipated for 1985, but a maximum of only 25,000 MW can be expected because of recent breakdowns in power stations and the postponement of various building projects. □

SWEDEN

● A report recently published by Sweden's National Environment Protection Board shows that the business of making polluted lakes clean is slow and difficult. Sweden has 100,000 lakes covering 9% of the country's surface, and of these about 15% in the southern third of the land are being overloaded with phosphates and nitrogen. In general, the polluted lakes are victims of acid rain, sewage, industrial effluents and agricultural run-off. Recovery programmes are having limited success.

In the long term, acidity will be reduced only if fuels can be burned without releasing much sulphur into the air; but as a stop-gap measure, lime has been spread onto the lakes themselves in some severely-affected areas. Prospects for large-scale liming seem poor, however, not only because of the cost but also because the traces of toxic metals found in most commercial lime would become significant in the quantities involved.

Another recovery tactic has been to cut off flows of effluents into a lake and the streams feeding into it. This has only improved water conditions slightly, as the lakes' internal circulation of nutrients has not been affected enough to cut down the production of plankton to acceptable levels. Reducing pollution by treating sewage to remove the nutrients from it is not always effective either: although it caused plankton levels to go down in one case, the gains were partly offset because the farmers in the area increased their use of nitrogen fertiliser.

In some cases, causes and effects in the pollution cycle are impossible to determine. The fact that the burbot in Lake Vättern has decreased dramatically in the past ten years while its main prey species has increased could be due to the burbot's

high PCB content or simply to over-fishing. But once the balance between the two species has been upset it may never be re-established, and the reason for the change may never be known. Pike in Lake Vänern are black-listed because they contain mercury from industry at the northern end of the lake. The fact that they have been fished less has meant that their average size has grown, which has resulted in increased cannibalism and a slower growth rate in their population. In these circumstances it is extremely difficult to say exactly what the direct effects of mercury pollution on pike may be.

With these uncertainties, recovery programmes are hard to design. The report gives the impression that the problem would be easier to tackle by prevention rather than cure.

● The Nuclear Fuel Supply Company has reached an agreement with the French firm Cogema under which spent nuclear fuel from the Barsebäck 2 and Ringhals 3 reactors will be reprocessed at the French facility in La Hague. Cogema will accept spent fuel produced until the end of 1979, and will return it after reprocessing to Sweden for final storage. Because of delays in the Ringhals 3 programme, it seems likely that the spent fuel sent will in fact come only from Barsebäck 2.

Under the law specifying the conditions to be met by reactor operators before the government will give permission for the building and operation of nuclear power plants, the owners of Barsebäck 2 must organise secure reprocessing of spent fuel. In their opinion the present agreement fulfils their obligation. Sweden's Energy Minister, Olof Johansson, who is trying to stop Sweden's march into the nuclear society, is refusing to admit

defeat. He says that the government can decide whether the owners have met the conditions only after it has examined the contract. The government's pronouncement is expected to be made by 1 October.

● According to a recent statement by the Minister of Education, Mr Jan-Erik Wickström, Sweden may be facing an acute shortage of people educated in technology and the natural sciences. The 1970s, said Mr Wickström, have seen difficulties in recruiting students to technical and natural science lines at both secondary and tertiary levels. He blamed this partly on the state of the labour market, which was unfavourable for natural science graduates at the beginning of the 1970s, partly on the evident inability of primary schools to exploit students' interest in natural sciences, and partly on the unpopularity of technicians and scientists in a society affected by the green wave.

A look at the official statistics hardly clarifies the picture. It is true that the number of students reading natural sciences and mathematics at tertiary level fell by 25% between 1971 and 1976. But that should be seen against a background of a general drop in interest in tertiary education: the number of students reading humanities and social sciences, for example, fell by 20% during the same period. Yet these years saw the number of students at institutes of technology increase by 9%. A similar pattern holds for doctoral degrees taken over the same period: down by 16% for mathematics and natural sciences, down by 17% for humanities and social sciences, but up by 5% for technology.

Wendy Barnaby

IN BRIEF

Nuclear study

In a declaration issued at the end of the two-day summit meeting in London at the weekend, the leaders of the world's seven leading non-communist industrial nations committed themselves to increasing nuclear energy to "help meet the world's energy requirements" and announced the launching of an "urgent study" (a "preliminary analysis, to be completed within two months", according to an annex) on how to do this while reducing the risks of nuclear proliferation.

The announcement, one of the main features of the final declaration, was carefully worded to smooth over the differences that have emerged within the western alliance since President Carter began acting on his worries about international trade in nuclear technology for ostensibly civilian purposes.

The proposed committee is expected

to consist of experts from the various countries and the study, on uranium supplies and aspects of the nuclear fuel cycle, will decide the terms of reference to be followed for a broader study lasting perhaps a year. Though it will be difficult for any such committee to agree conclusively, particularly in so short a time on what is now so political a matter, the key point is itself political, namely that the study represents a review of nuclear policy at the highest possible political level.

Geos update

Initial testing of experiments on board ESA's research satellite Geos has now been completed; all were working well early this week. Final operating conditions were due to be achieved by mid-week when the long radial booms would be fully deployed to 20 m. The booms had been at 2.5 m, giving a rather high spin rate for the particle

experiments. The slow drift of the apogee to longitude 37.7°E was halted on 8 May and Geos is now in an orbit with apogee conjugated to Northern Scandinavia, but a limited programme of correlated measurements will be possible.

The satellite is in view on every second 12-hour orbit for nearly 8 hours per day and the viewing time is well matched to the period during which the altitude is greater than the 3.5 Earth radii where all experiments can operate. The viewing time could be doubled by collecting data from the other orbit using NASA ground stations in Alaska, but the apogee remains at the same local time. It will be next February before the local time of the apogee moves from early evening through the day to probe the night-side region of the magnetosphere.

NASA's investigation into the failure of the launch vehicle continues. A detailed report is expected this week.

ON 30 April the blown-out Ekofisk Bravo oil well in the middle of the North Sea was finally capped, having spewed 12,000 tons of oil into the sea in the previous nine days. Three days later the oil, constantly tracked by satellite, had virtually disappeared. Such a startlingly anti-climatic ending to a drama billed as an ecological disaster must leave a few people somewhat puzzled as to where it all went.

A good deal of it of course went upwards. The UK Department of the Environment in *Accidental Oil Pollution of the Sea* (Pollution Paper no. 8, 1976) estimates that up to 40% of the relatively light North Sea oil may evaporate in one day; oil from the Ekofisk field has an even lower specific gravity than the North Sea average. The volatile fraction fortunately includes the most toxic components of the oil, the aromatic hydrocarbons such as benzene and toluene.

What was left after evaporation and solution formed that water-in-oil emulsion known as 'chocolate mousse' whose persistence normally causes great despondency. But in this case, probably again because of the lightness of the oil, the emulsion was an unstable one, quite easily dispersed by the rough seas which had conveniently developed soon after the well was capped. The silver sheen slick of dispersed oil droplets covered a huge area for a short time, but by the middle of last week was reduced to a few square miles.

Once the oil had been broken down into small droplets the paraffins could be digested by bacteria. Some particles are ingested by zooplankton which die and fall to the seabed, making the oil available to the more abundant bottom-living bacteria. The

Where goes the oil?



BACKGROUND

enormous amount of extra carbon available will cause short-term changes in the community but probably no long-term disruption. Though normally one would expect some tar to be formed from the heavier residues there may be virtually none from the Ekofisk oil.

Nature was responsible for 90% of the oil dispersal; the rest was managed by the Norwegians, who contained the oil with booms and

managed to skim 1,000 tons off the surface. A better figure probably could not have been hoped for: removal of the oil is certainly the ideal solution to the pollution problem but present equipment, being ineffective when waves are more than 7-foot high, is useless for most of the time in the very rough North Sea. The clean-up ships were fortunate in this case to get two days of near calm to pump most of the thicker patches of oil off the sea.

By yet another happy chance the blow-out happened at such a time as to cause minimal damage to fisheries and birds. The Ekofisk field is in the middle of Norway's valuable mackerel fishing ground; mackerel do not swim near the surface but their eggs, which are laid during May, float near the surface and would certainly have been damaged if the blow-out had happened three weeks later. The Norwegians are reluctant to use chemical dispersants on oil because of their toxic effects on marine life. In this case they were proved right by the rapidity with which the oil disappeared but there would have been strong pressure to use chemicals if the accident had happened in July, when large numbers of auks are swimming across the North Sea from Scotland to Norway.

Thus, apart from a few unanswered questions, for example concerning the long-term effects of hydrocarbons in food chains, there is cause for great relief. Things may not work out quite so well next time.

CEGB change

The retirement of Sir Arthur Hawkins as chairman of the UK Central Electricity Generating Board (CEGB) at the end of last week has raised the prospect of an early resolution of the problem which the proposed Drax B coal-fired power plant poses. The issue is not whether it should be ordered, but when. That depends in the first instance on electricity demand, but other factors have intervened.

The plant was planned for 1979. The turbine and boilermaking industries face a lean time with demand down and want the order brought forward; the government's 'think tank' has recommended a rationalisation of the industry and this is still under discussion. The CEGB has said the order can be placed now, but wants compensation from the government. What is in dispute is the amount of that compensation, and who should pay it. The hope is that with the departure of a key

figure in the growing controversy, some new formula can be found to satisfy Glyn England, the new CEGB chairman. With the visit of Mr Benn, the Energy Secretary, to Moscow this week for talks with Mr Kirillin, his Soviet counterpart, no announcement is expected before next week.

On the move

Dr Frederick Horner is to take over from Dr J. A. Saxton as Director of the Appleton Laboratory near London. Horner joined the Radio Division of the National Physical Laboratory in 1941, which has since evolved into the Appleton Laboratory. In 1969 he became Deputy Director. Most of his research has been concerned with finding radio direction and studying radio emissions from thunderstorms. Since 1950 he has been involved in international radio affairs and has held office in international organisations. Saxton retires on 1 July.

The UK Department of Health will soon be losing its Chief Scientist, Sir Douglas Black, following his election as President of the Royal College of Physicians. No replacement has been announced.

WHO message

The World Health Organisation (WHO) should strive to achieve a universal level of health conducive to high social and economic productivity by the year 2000. This was the message of Dr H. Mahler, Director-General of the WHO, when he presented his report for 1976 to the World Health Assembly in Geneva last week. He said that the WHO should through its country health programming promote development in primary health care and appropriate health technology, even though in so doing it might meet with opposition from the medical and various allied professions and industries.

In Britain, most western European countries and the United States, the so-called 'organic movement' is growing. Its members believe that 'natural' manures, derived from animal excreta or composted vegetable matter, are better than factory-made chemical fertilisers. The movement embraces those with widely different views. The more extreme believe that chemical fertilisers poison the land and those who eat its products. The more moderate consider that organic manures improve the structure and fertility of the soil better than do inorganic fertilisers, and produce crops which differ nutritionally in subtle ways from those grown with liberal dressings of artificials. They claim that pests and diseases are less rampant when organic techniques are used, though they admit that the highest yields are often obtained with balanced dressings of inorganic fertilisers and the liberal use of chemical pesticides. In general they claim quality rather than quantity for organic produce.

As an entomologist on the staff of Rothamsted Experimental Station, on whose fields the classical experiments demonstrating the value of chemical manures were performed, I was at first very sceptical. However, when, over twenty years ago, I visited the experiments of the Soil Association at Haughley in Suffolk, and examined the long-term experiments set out and organised by Lady Eve Balfour, I decided that this work needed to be looked at critically and scientifically. I did not have an experience like St Paul on the road to Damascus, with an instant conversion, and I found

some of the explanations of organic farmers and gardeners hard to accept, but I did think that this unique long term study, where comparable fields were receiving different fertiliser and pesticide treatment, ought to be thoroughly investigated.

I attempted to interest bodies like the Agricultural Research Council,

Muck is magic



KENNETH MELLANBY

who were at that time comparatively generously supplied with funds to support research. I could not persuade them to part with a penny to investigate the work at Haughley, or to study organic methods generally. Time and time again my advances were rebuffed. Even when I got together a Research Advisory Committee, which included hard-headed

scientists from the chemical industry, no government money from agricultural research funds was forthcoming. In fairness to the ARC and my former colleagues I must admit that they believed that the subject had been thoroughly studied by established methods, and it was not unreasonable to assume that little more was to be discovered. Furthermore, some of the extreme organic fanatics talked such evident nonsense, and their more rational colleagues suffered by association.

Today things seem to be different. I was delighted to see a headline in the *Times* recently which read "Ideas of organic farming being taken more seriously", and to discover this referred to a lecture by a member of the staff of the ARC. Not long before I heard my former colleague Dr G. W. Cooke, recently the distinguished head of the Chemistry Department at Rothamsted and now also with the ARC, admit that modern work had shown that organic manures were now being found to increase some crop yields much more than would have been expected from an analysis of the inorganic nutrients present.

I hope that this will mean that organic methods will now be really critically studied, as I proposed so long ago. If the ARC sees the light in this way, they may wish to follow the example of the now-defunct Council for Scientific Policy. This body prefaced its last report with an extract from the General Confession in the Book of Common Prayer. I suggest that the ARC choose as its text Luke 15, v. 7.

correspondence

'The Selfish Gene'

SIR,—Lewontin's review of Dawkins' book *The Selfish Gene* (17 March, page 283) is a disgrace. It fails to meet any of the standards of informative value, objectivity and fairness to the views of others that are part of the code of science. That it is a very biased review is reasonably evident to anyone reading it; unfortunately a reader unacquainted with the controversy which is its background may well be left with the impression that, even setting aside obvious unpleasantness in the review, the book itself probably is unsound and not worth reading. This is a great pity since in fact the book is not only the best existing outsider's introduction to a new paradigm and a new field of knowledge but, in its overview of the situation and in many original details, is itself a significant contribution to this field.

For its intellectual worth, and seemingly in motivation as well, Lewontin's outline of the book is on par with Bishop Wilberforce's notorious attack on Darwin and Huxley at the British Association meeting of 1860. It would be easy to defend the book and reply to the review point by point, but this would be an unpleasant, muck-raking task, and it would be difficult to avoid matching rhetoric against rhetoric and so bringing debate to the plane where Lewontin wants it to be. Against such a review I feel justified in following Huxley to give a very subjective assessment of my own but wish to avoid trying to match the wit of the historic reply to the bishop.

Whether the literature of socio-biology reflects an ignorance of the difference between "properties of sets

and properties of their members" or "confusion between materialism and reductionism" are matters about which I feel little concerned. On the other hand I feel a warmth very far from indifference when I encounter in most of the literature in question signs of a spirit which I share and which I have always assumed is the same as that which motivates scientific enquiry in all its branches. I can most simply express this spirit by calling it a desire to understand and communicate the nature of the world. I find it present in full measure in *The Selfish Gene* and totally absent in Lewontin's review.

Yours faithfully,
W. D. HAMILTON

Imperial College Field Station,
Ascot, UK

Efficient fish

SIR,— "... fish are not efficient users of food materials other than animal protein and fat", according to Dr Purdom and Mr Preston (31 March, page 396). This is incorrect for some species. At Reading we routinely obtain feed conversion ratios of less than 10 kilograms of fresh vegetation to 1 kilogram of fish when feeding ryegrass (*Lolium* spp.) to grass carp (*Ctenopharyngodon idella* Val.). This represents a protein conversion efficiency of 40%. Equally efficient conversion of terrestrial vegetation is recorded in the literature for *Tilapia melanopleura* Dumeril and *Tilapia zillii* Gervais in pond culture. The figure of 100 to 1 given by Purdom and Preston is applicable to a diet of aquatic vegetation which consists largely of water. Moreover, we have evidence which suggests that leafy ter-

restrial vegetation provides all the major nutrients required to support rapid growth rates in grass carp.

If it is assumed that ryegrass for artificial drying costs £6 per tonne fresh weight, the feed cost is 6p per kilogram of fish produced. Feed costs could be reduced by using agricultural by-products and food-processing wastes. It should therefore be technically possible to farm fish intensively and at low feed costs. Farmed herbivorous fish are rated highly as food throughout much of Asia, Africa and Eastern Europe.

Yours faithfully,
J. C. J. DOMANIEWSKI

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Horticulture,
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Value of textbooks

SIR,—During recent discussions on the structure of the living cell at meetings of the Physiological Society, the Anatomical Society, the Biochemical Society, and the Royal Microscopical Society, several participants have made the remark "Surely, you don't believe what is written in text books". This viewpoint seemed to be held by several other participants at the meetings. This raises such fundamental questions of scientific truth, that we wonder if any of those who support this view might be prepared to justify it in print. We should say that we were grossly disturbed by such a suggestion and thoroughly disagree with it.

Yours faithfully,
H. HILLMAN
P. SARTORY

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Health,
University of Surrey, Guildford, UK



Competition 13

(From an idea by Martin Rees). In the next few years we discover—never mind how—that there is intelligent life nearby in the universe, but that we are more 'advanced' than they. A prize of £10 is offered for the best short message to their equivalent of

Isaac Newton, or any other character from our history, telling him what scientists are up to these days. Send entries by 15 June to the Editor, and mark envelope Competition 13.

Competition 12 asked for fatuous, actual or fictitious professorships. Honourable mention to Drs Haslam and Haslam for the fictitious Chair of Wind Engineering in the Medical Faculty (to go with a new journal entitled Wind Engineering) and the actual Chair of Artificial Intelligence.

But the prize to Professor V. Trimble (Irvine) for the following:

It would be difficult to top Cambridge's own (real) Plumian Chair of Astronomy and Experimental Philosophy, whose incumbent, we presume, is required to go about kicking stones and looking for trees in quads where there is no one about to observe them. Recent interest has, however, also been attracted by Brown University's (fictitious) chair of Psychoceramics, whose present holder, Professor Carbury, is, of course, an expert on cracked pots. My own university's English department has contemplated a chair in Historical Metallurgy to be reserved for someone learned in the study of Old Saws.

news and views

Cosmic gamma-ray bursts

from K. J. Orford

SINCE their first reported discovery 4 years ago, cosmic gamma-ray bursts have been observed at a rate of roughly one per month. These events have until now been observable only from satellites, or balloons, since the weak gamma-ray energy flux cannot penetrate the atmosphere or compete with atmospheric radiation. Their theoretical interpretation has been hindered by a lack of sufficient knowledge of their celestial positions, their distribution in size, and a lack of any correlated phenomena which could narrow the search for sources. As a consequence the number of theoretical models has exceeded, if not the number of bursts (about 50), then at least the number of well established parameters. Some recent results reported in *Nature* may help.

Manchanda and Ramsden (*Nature* 266, 425; 1977) point out that about 90% of the bursts have sizes, or energy fluxes, within a relatively narrow range. If the assumption is made that they all originate in explosive events of similar energy output then a burst's size reveals its distance, at least relatively. The distribution in distance so obtained bears a remarkable resemblance to the distribution of supernova remnants. Supernovae are thought to be sources of both neutron stars and black holes. The disruption of a neutron star by a black hole would provide a neat explanation not only of the size distribution, but also of the gamma-ray burst's energy spectrum. Two consequences of this theory are that the bursts should have a particular distribution in celestial position and that very small and very large bursts should be relatively infrequent. The first must await the accumulation of a large number of bursts with well measured positions, easy only for the rarer large bursts which are recorded by several satellites. The second is in contrast with the situation for many random astronomical phenomena, for which the count rate is in some way inversely dependent on size, that is,

smaller bursts would be expected to be more frequent. Smaller bursts are indeed observed to be less frequent, but unfortunately just at a size where current satellites have difficulty in detecting them.

To resolve this, a number of balloon-borne experiments have been carried out with very large detectors. Such detectors, some hundred times larger than those at present carried in satellites, should be capable of seeing bursts about two orders of magnitude smaller in size. Their lack of confirmed observations has enabled some upper limits to be placed on small burst rates, with consequences for burst origins (for example Carter *et al.* *Nature* 262, 370; 1976). However Cline *et al.*, at the Goddard Space Flight Centre, have revealed a source of confusion for such experiments (*Nature* 266, 694; 1977). In an attempt to detect very small bursts, and to prove that any bursts seen were of cosmic origin, two similar detectors were flown on balloons separated by at least 1,400 km. They achieved 20 h of simultaneous observations, but detected no coincident bursts of cosmic gamma rays. Slow 'bursts' were observed on each individual payload and some were associated, but separated in time by many seconds. Whatever the cause of these (suggested to be due to magnetospheric activity), they constitute a serious source of background noise for burst experiments using a single balloon or near-Earth orbit satellite. As these authors point out, much more sensitive experiments are needed to enable small bursts to be seen and their expected anisotropy to be observed.

It may be that the theoretical interpretation of bursts may be helped more immediately by the observation of some correlated phenomenon. One particular burst was observed on 16 August last year by one of the chain of Vela satellites responsible for the original discovery, by two new satellites, Solrad 11A and 11B (Laros *et al.*, this issue of *Nature*, page 131) and by a transatlantic balloon experiment. The gamma-ray results published do not by themselves mark out the burst as very special (except as a simul-

taneous observation by a number of different experiments, including a balloon flight). However, what may be a very significant radio observation was made by Mandolesi *et al.* (*Nature* 266, 427; 1977) at Medicina and Trieste. They observed on 16 August 1976 a radio burst at three widely spaced frequencies at Medicina, and at a fourth frequency using a 10-m parabola at Trieste. All lasted about 30 s, the duration of the gamma-ray burst, and they rule out man-made, atmospheric and solar effects as its cause. It occurred less than one minute after the gamma-ray burst, to which coincidence they ascribe a chance probability less than 10^{-4} . If the satellite burst position is eventually found to agree with the radio position, it will confirm the first such burst-correlated phenomenon. The separation in time would then be a consequence of the evolution of the burst source and would limit the models proposed to explain both bursts. Since this coincident radio burst was discovered only about a month after full operation of the radio array, and if the two bursts had a genuine common source, then one may hope that further coincidences will soon follow. □



A hundred years ago

THE WOODPECKER.—In the April session of the German Ornithological Society Prof. Alton concluded the recital of his investigations on the habits of the woodpecker. The peculiar drumming sound often caused by it was shown on various grounds to be entirely disconnected with the search for insects as hitherto supposed, and was regarded as a call to the opposite sex. Dr. Brehm defended the woodpeckers against the charge of seriously injuring the trees, and considered the slight damage resulting from them as more than compensated by the colour and animation which they gave to the otherwise sober and quiet forests.

From *Nature* 16, 10 May, 30; 1877.

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Sequencing DNA

from Maria Szekely

It is somewhat unusual that a new technique should already be being used all over the world at the time of its publication, but this is exactly what has happened with the ingenious new method of DNA sequencing worked out by A. Maxam and W. Gilbert at Harvard University. The method is now described in full detail (*Proc. natn. Acad. Sci. U.S.A.* **74**, 560; 1977), but already DNA sequences established in this way have been published—by Walz *et al.* (λ promoter-operator region (*Nature* **262**, 665; 1976)), by Fiers' group (SV40 DNA (*Cell* **9**, 117; 1976)), by Musso *et al.* (control region of the *gal* operon (*Proc. natn. Acad. Sci. U.S.A.* **74**, 106; 1977)), by Sures *et al.* (histone genes (*Cell* **9**, 495; 1976)) and others, showing how a useful technique can spread at amazing speed among scientists working in this field.

DNA sequencing, which some years ago seemed a formidable task and was approached by trying to convert it to an RNA sequencing problem (Gilbert and Maxam themselves sequenced the *lac* operator in 1973 (*Proc. natn. Acad. Sci. U.S.A.* **70**, 3581) by transcribing it into RNA and determining the sequence of this transcript) is today a relatively simple operation which can be accomplished by routine methods much more quickly than RNA sequencing could ever be done. In fact, the present trend is to convert RNA sequencing problems into DNA sequencing tasks to speed up the work.

Two very efficient techniques are available today to establish DNA sequences: Sanger's 'plus and minus' technique (*J. molec. Biol.* **94**, 441; 1975), based on enzymatic copying of single-stranded DNA, the spectacular results of which have recently been published in *Nature* (see **265**, 687; 1977) and Gilbert and Maxam's technique based on chemical modification and breakdown which has been applied to a variety of double-stranded DNA fragments. Both methods have in common a very convenient and fast way of interpreting the data: the sequence can be read off the gel electrophoretic pattern obtained from a complete mixture of different sized fragments. This readout technique makes it possible to establish long sequences in one experiment; the longest nucleotide stretches which can be analysed are in the range of 100–150 nucleotides and

the upper limit depends only on the resolution attainable in the gel electrophoresis.

The basic principles of the two methods are quite different however. Sanger, in the 'minus' technique, copies a single-stranded DNA stretch, synthesising different length fragments, each of which ends before a given base. Running four parallel samples in which stoppages occur at different bases provides sufficient information to read the sequences of the whole DNA fragment. The 'plus' technique serves to confirm the sequence; here the fragments are degraded by making use of the exonuclease activity of T4 DNA polymerase, under conditions when this degradation stops at sites where a given base is present. Again, four samples are run in parallel to determine the positions occupied by all four bases in the DNA fragment.

In the technique of Gilbert and Maxam no copying is involved, nor any enzymic reactions. Chemical reagents are used to modify and excise a given nucleotide from the polynucleotide chain. If conditions are mild, only partial modification occurs and only about one in 50 or 100 nucleotides is excised. In a random mixture there will be fragments of different lengths ending at all possible positions where this base was present. Four parallel samples are prepared also in this method to locate the positions of all four bases along the DNA chain.

In many cases either method can be used successfully for DNA sequence determination. The choice of the appropriate technique depends mainly on the single or double-stranded nature of the DNA. Sanger's technique requires single-stranded DNA whereas the Gilbert–Maxam procedure works equally well with single and double-stranded molecules. One variant of the Gilbert–Maxam procedure involves denaturation of the DNA fragment and separation of the strands, this step can, however, be omitted and another strategy used instead; the 5'-termini of a double-stranded DNA fragment can be labelled and the fragment split in the middle. This treatment yields two smaller fragments in each of which only one strand carries a labelled group. Consequently, only the products from this strand will show up on the autoradiographs. This represents a great advantage, as strand separation may prove difficult when applied to longer DNA molecules (although the Gilbert–Maxam separation method seems to work very well for restriction

fragments). As to the length of the DNA molecule to be sequenced, the Gilbert–Maxam technique has been mainly used so far for determining the sequence of restriction fragments and is probably best suited for fragments 100 to 200 nucleotides long. In the plus and minus technique, copies of any desired length can be synthesised from a convenient and well-defined start point, irrespective of the length of the DNA template. The method has also been used on the intact complete genome of ϕ X 174 phage (Sanger & Coulson *J. molec. Biol.* **94**, 441; 1975). The start point is determined by the oligonucleotide used for priming the synthesis of the labelled copy and can thus be chosen more or less at will.

Apart from the very great progress in elucidating the structure of different genomes, these techniques may well prove of great advantage in the field of RNA sequencing. Complementary DNAs have already been produced for sequence studies on eukaryotic mRNAs (see for example Proudfoot *J. molec. Biol.* **107**, 491; 733; 1976 and Cheng *et al. ibid.* 527). These cDNAs may now be accessible to the new sequencing techniques. We may expect in the near future a great advance in the sequence analysis of this group of mRNAs which are of great functional importance but which so far have presented serious problems for sequence studies. □

Man-made auroral pulsations

from Alan Johnstone

PULSATING auroras are one of the most intriguing types of auroral display. Irregularly shaped patches of light appear to turn on and off regularly with a period of the order of 10 s. The beam of electrons, which produces the auroral light when it strikes the atmosphere, must be modulated by some plasma wave, on its way down the magnetic field line from the outer radiation belt. Despite much speculation about the cause no satisfactory explanation has been found. The report

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An important control on the immune response in many animals, including rodents and primates, appears to be provided by the immune response (Ir) genes which are tightly linked to the major histocompatibility complex (MHC). These genes are antigen specific in their effects, that is, they appear to play an important part in determining whether an animal can respond to any particular antigen, but as yet their function and site of action is still unclear. However, evidence is rapidly accumulating that the Ir genes are in fact expressed in macrophages or the macrophage-like cells that process or present antigen to T lymphocytes.

Rosenthal and Shevach (*J. exp. Med.* **138**, 1194, 1213; 1973) showed that guinea pig T cells from an F_1 hybrid between a responder and a non-responder could only be stimulated to proliferate by antigen-coated responder (or F_1) macrophages, but not by non-responder macrophages. Subsequently, analogous proliferative studies have been performed in the mouse by Schwartz and his colleagues (*J. Immun.* **117**, 531; 1976 and *Proceedings 3rd Ir Gene Workshop*, Asilomar, 1976). These studies suggested that the proliferating cells recognised antigen in association with macrophage Ir gene products, which since they were inhibited by anti-Ia antisera (antisera directed against the I region-associated antigens of the mouse MHC) were probably macrophage Ia antigens. While the immunological role of the proliferating cell(s) in these assays is not known, analogous results have been obtained in assays for the induction of antibody (Pierce *et al.* *J. exp. Med.* **144**, 371; 1976) or of helper cells (the T cells which collaborate with B cells to enable the latter to produce antibody). For example, Erb *et al.* (*Eur. J. Immun.* **6**, 365, 1976) have demonstrated the capacity of macrophage Ia

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Are the Ir genes expressed by macrophages?

from Marc Feldmann

antigen, complexed to a fragment of immunogen, to form a soluble complex of molecular weight ~55,000 daltons, which replaces intact macrophages in the induction of helper cells. All these studies reinforce the concept that there are Ir gene effects expressed in macrophages. J. E. A. P. Miller and his colleagues have recently obtained compatible results in a delayed hypersensitivity (DTH) model in the mouse, leading to the same conclusion for the Ir genes involved in DTH (Miller *et al.* *J. exp. Med.*, in the press).

In this issue of *Nature*, Rosenthal, Barcinski and Blake (page 156) extend this point. Using insulin molecules from various species, with minor differences in amino acid sequence, they demonstrate Ir gene control of antigen recognition at the level of the macrophage, measured by the proliferative response of guinea pig T cells. Their results are most easily interpreted by postulating that macrophages may select the appropriate portion of the antigen to be presented to T cells. How is this done? The simplest possibility is that macrophage Ir genes define a class of receptors of broad specificity that 'focus' or orient antigen for T cells to recognise it. Conceivably, these receptors may be the descendants of the recognition molecules which antedate the vertebrate immune system. If that interpretation is correct the antigen-presenting cells, and not the T cells, dictate the antigen specificity of the Ir gene effect. But since T cell responses depend so intimately on macrophage presentation, macrophage Ir genes would reveal them-

selves (as has been found) as defects of T cell function. The search for the immune response gene products may then already be partly over—they have been isolated and partly purified as the macrophage Ia antigen-immunogen complex, which induces the activation of helper cells *in vitro*.

The idea that the Ir genes are expressed on macrophages is also compatible with other findings that have been interpreted quite differently. Munro and Taussig for example (*Nature* **256**, 103; 1975), described two types of mice with genetically determined low responses to the synthetic polypeptide (T,G)-A-L; those which did not make T helper factor (an antigen-specific mediator of T cell help) and which was interpreted as a T cell defect, and those which did not respond to T cell helper factor, which was interpreted as a B cell defect. But since macrophages are needed for both these reactions a macrophage defect could be at the root of both these failures. T cell suppression has also been proposed as a mechanism of non-responsiveness and since suppression is much less dependent on macrophage function than T cell help, a net suppression would occur in the presence of dysfunctional macrophages. This idea is not incompatible with the proposed role of the macrophage.

Whatever the truth of the mechanism of Ir gene action, it now seems certain that macrophages and macrophage-like cells have a more complex role in the immune system than hitherto suspected, and the precise nature of the antigen-presenting cell needs to be re-examined. Are these classical macrophage or lymphocyte-like cells, or a hybrid between the two. In view of the plethora of lymphocyte subpopulations recognised in the past two years, the existence of yet another type of cell in the immune system need not be too improbable. However, the question still remains as to whether there are Ir genes expressed by other cells of the immune system.

that pulsations have been induced artificially near a pulsating display is unexpected and might give valuable insight into the mechanism causing the natural phenomenon.

The artificial pulsations were found by Deehr and Romick (this issue of *Nature*, page 135) in one of the natural auroral spectral lines at 200 km altitude, just below a barium release in the F region of the ionosphere. This technique, in which 1.5 kg of Ba is ejected by a thermite reaction from a canister carried aloft by a rocket, is used to measure electric fields and

winds in the upper atmosphere. The barium cloud is partially ionised by sunlight, forming a cloud of ions which resonantly scatters purple light from the Sun and a neutral cloud which scatters green light. The neutral cloud is blown across the sky by winds while the ionised cloud is driven by the combination of electric fields and winds. By tracking both clouds optically from the ground the effects of the two forces can be separated. The technique is very effective and has been used many times in the last decade.

Ideally, the barium should not dis-

turb the medium it purports to measure. Small barium releases, such as that used by Deehr and Romick, do not distort the electric fields seriously although they must have some effect. Their observation is not the first of perturbations in the ionosphere beneath a barium cloud. Stoffregen (*J. atmos. terrest. Phys.* **32**, 171; 1970) detected a temporary increase in the auroral line at 557.7 nm at 100 km altitude below a barium cloud. He did not observe pulsations and there was apparently little natural aurora present. Spracklen and Jones (*Q. Jl R. Astr.*

Soc. 17, 469; 1976) have observed an increase in E region (100 km) electron densities by radio techniques. Both authors have attributed this coupling between the F region where the barium was released, and the E region where the disturbance is observed to the flow of charge from one side of the cloud to the other to neutralise the build up of a polarisation electric field as the cloud starts to move. The horizontal current, across the magnetic field, can only flow in the E region where there is sufficient Pedersen conductivity to allow it. The downward flow of electrons from one side of the cloud enhances the electron density in the E region while the upward flow on the other side should leave a lower density.

The release reported by Deehr and Romick is the only one to take place within a significant amount of natural aurora and its presence seems to be a necessary condition for the pulsations. They were observed at 200 km where electrons of 500 eV deposit most of their energy. Higher energy electrons would penetrate further into the E region, but any effects there might have been were masked by natural aurora.

Natural pulsating auroras are found

predominantly at altitudes below 100 km (Brown *et al. Geophys. Res. Lett.* 3, 403; 1976) implying that higher energy electrons, between 10 keV and 60 keV, are usually involved. Bryant *et al. (Planet. Space Sci.* 23, 867; 1975) measured the electron fluxes producing pulsating aurora directly and also found relatively high energy particles. Further, they were able to detect phase differences between the pulsations observed at different energies, with the higher energy pulsations seen first. This velocity dispersion placed the source of the modulation more than 40,000 km away, and certainly not within 300 km.

The differences between what is known of natural pulsations and the circumstances surrounding the observations of the artificial ones seem to suggest that the existence of the two displays side by side was coincidental. It would be hasty to draw such a conclusion when so few hard facts are known about either phenomenon and when the range of possible mechanisms is still so wide. One thing is certain—there will be determined attempts to repeat the observation during future barium release experiments wherever they are performed. □

It seems likely that complex interactions between peptidergic neurones will be uncovered since neurones originating in dorsal root ganglia and separately containing Substance P and somatostatin enter the spinal cord where they overlap with neurones containing enkephalin, neurotensin and TRH. Peptide-monoamine interactions also seem likely in view of the high density of enkephalin- and Substance P-containing neurones in areas containing cell bodies for serotonin, dopamine and noradrenaline. Iversen reported experiments that indicate an inhibitory control of Substance P release by GABA, and raised the possibility that these putative transmitters represent excitatory and inhibitory controls respectively for dopaminergic mechanisms in the basal ganglia. This may have important consequences in our understanding of various disease states such as Huntington's chorea and Parkinson's disease.

New results and controversy still abound in the opioid peptide field. The potent analgesic and behavioural effects of β -endorphin are the subject of considerable research (D. de Wied (University of Utrecht) and D. G. Smyth (National Institute for Medical Research, London)) and now it seems that stable analogues of enkephalin produce similar effects, even on intravenous administration, according to reports by R. Miller (Wellcome Foundation) and A. Z. Rónai (Budapest). Unfortunately there is little evidence yet that these short chain peptide analogues will prove to be less addictive than morphine. A promising note was sounded by L. Terenius (University of Uppsala) who described the possible anti-schizophrenic action of the opioid antagonist naloxone in Swedish patients, thus implicating endorphins in some mental disease state. Caution was sounded here, however, by the report that American psychiatrists had failed to reproduce these results. Terenius also finds increased levels of endorphins in the cerebrospinal fluid of schizophrenic patients and results in this area may prove easier to replicate than in the more problematic area of the clinical assessment of schizophrenic remissions. A further link between analgesia and endorphins was reported by Terenius and J. Hughes (University of Aberdeen) who both found increased opioid peptide levels in the cerebrospinal fluid of patients receiving intracerebral brain stimulation for the relief of chronic pain.

The major impression left by this symposium was the impressive accumulation of evidence for central peptidergic transmission but the lack of evidence relating to defined physiological roles. However, most par-

Centrally active peptides

from John Hughes

A symposium on Centrally Active Peptides, organised by the Biological Council, was held at the Middlesex Hospital Medical School, London, on 4-5 April, 1977.

AN extra dimension has been added to neurobiological research in the past few years with the recognition of the potential importance of peptide-releasing neurones in the brain. The concept of peptidergic neurotransmission and neuromodulation has reached fruition as an area of multidisciplinary research which promises major advances in our understanding of normal and abnormal brain function.

Important advances in the distribution of peptides were reported and the great value of immunohistochemical techniques in this field was emphasised. A picture emerged of an extensive distribution of peptidergic neurones throughout the brain and spinal cord. Prominent amongst these are neurones containing Substance P and enkephalins; these systems seem to rival and perhaps exceed that of the better known catecholamines. A significant advance has been the demonstration of

cell bodies for peptidergic neurones (R. P. Elde (University of Minnesota), S. H. Snyder (Johns Hopkins University) and L. L. Iversen (MRC Neurochemical Pharmacology Unit, Cambridge)). It seems that the enkephalins are contained in short interneurones with a terminal distribution closely paralleling that of opiate receptor binding sites. This system appears to impinge on areas receiving primary sensory inputs as well as on those involved in the control of respiratory, cardio-vascular, somatic motor and neuroendocrine functions.

Iversen presented cogent evidence for a central neurotransmitter role for Substance P, and this peptide is now a firm favourite as one transmitter involved in the relay of information by small primary afferent sensory fibres. Multiple transmitter roles for Substance P also seem likely from its distribution in other areas such as the basal ganglia, hypothalamus and limbic system. Other neuronal systems containing vasopressin, oxytocin, vasoactive intestinal peptide, thyrotropin releasing hormone (TRH), somatostatin and neurotensin have also been identified in many brain areas (Elde, M. J. Brownstein (National Institute of Mental Health, Bethesda), A. G. E. Pearse (Royal Postgraduate Medical School, London) and Snyder).

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ticipants seemed confident of finding further evidence for the participation of peptides in normal and abnormal brain function. Continuing research is likely to lead to a deeper understanding of neuronal physiology with corresponding therapeutic benefits. □

Membranes and receptors

by Peter Newmark

A conference on Receptor-Signal Transmission was held in Titisee on 30 March–3 April, 1977. It was sponsored by the Dr Karl Thomae GmbH, a subsidiary of Boehringer-Ingelheim.

A CLUSTER of receptorologists, convened for three days in the Black Forest, pondered not only the mobility of receptors but also the transmembrane activation of adenylate cyclase, mechanisms of cell adhesion and membrane changes in secretion.

The presentation of a ligand to its receptor may be an event of considerable complexity involving cooperative effects whereby the initial binding can either promote or hinder further binding. One of the best examples of this is the negative cooperativity demonstrated in the binding of insulin to its cell surface receptors. P. de Meyts (National Institutes of Health, Bethesda) was cautious in his discussion of the mechanisms of negative cooperativity. He has however obtained evidence that the avian insulin receptor still exhibits negative cooperativity when solubilised. The solubilised receptor exists in high molecular weight form in the absence of insulin, and is converted, reversibly, to low molecular weight form by addition of insulin. From that de Meyts suggests that negative cooperativity, often supposed to be the result of clustering of receptors, instead involves the dissociation of a high affinity tetramer form of the receptor to a low affinity monomer. de Meyts has also been looking for negative cooperativity with a series of insulin analogues and the results, interpreted in conjunction with the known three-dimensional structure of insulin, indicate that cooperativity is determined by a discrete domain on the surface of the insulin molecule distinct from that involved in binding.

A highlight of the meeting was the general, probably unprecedented, agreement that emerged from the various contributions on the molecular inter-

actions involved in the transmembrane activation of adenylate cyclase following the interaction of ligand with receptor (M. Rodbell, NIH, A. Levitzky, University of Jerusalem, E. Helmreich, University of Wurzburg and M. Schramm, University of Jerusalem). All agreed that the receptor is not physically coupled to the adenylate cyclase moiety and that the activation of the enzyme as a consequence of the ligand interacting with its receptor is a GTP-dependent process. The GTP, whose origin is intracellular, is thought by Rodbell to activate both the receptor and the adenylate kinase each by way of a separate nucleotide binding site. Levitzky favours the view that only the enzyme is activated by GTP. That the GTP activation is normally transient and is terminated by hydrolysis of the nucleotide is shown by the permanent switching on of adenylate cyclase by GTP analogues that resist hydrolytic degradation. Some progress was reported in the purification of the relevant GTP binding proteins (Rodbell and Helmreich) and of both the β -adrenergic (Levitzky) and epidermal growth factor receptors (C. F. Fox, University of California, San Francisco).

From the general agreement that

there was no direct, permanent association between receptor and enzyme, it can be predicted that a receptor from one cell should be able to couple functionally with the adenylate cyclase from another cell when the two cells are fused. Schramm reported experiments which bore out that prediction. In initial experiments (*Proc. natn. Acad. Sci. U.S.A.* **73**, 4410; 1976) Friend leukaemia cells, which do not possess β -adrenergic receptors, were fused with turkey erythrocytes which had been pretreated with N-ethylmaleimide or heat to inactivate their adenylate cyclase (not their receptor as mistakenly stated in *Nature* **265**, 681; 1977). Ghosts of the fused cells exhibited a low basal adenylate cyclase activity that was markedly activated by the β -adrenergic drug isoproterenol. Since then equivalent results have been obtained with mixtures of quite different cell types. For example the β -adrenergic receptor of rat C6-glioma cells can be coupled with the adenylate cyclase of mouse Y-1 adrenal tumour cells. What is more the affinity of the receptors for various agonists is unaltered after being coupled to a foreign adenylate cyclase.

I. Pastan (NIH) reported a series of studies that have led him to the view

Multinucleon transfer reactions

from P. E. Hodgson

MULTINUCLEON transfer reactions are now used extensively to study nuclear structure, and recently a group at Los Alamos has found remarkable similarities between the spectra of states excited in the same final nucleus by the ($^6\text{Li},d$) reaction on the one hand and by the (t,p) and (h,n) reactions on the other (*Phys. Rev. Lett.* **38**, 587; 1977).

The group measured the ($^6\text{Li},d$) reaction on ^{56}Fe , ^{57}Fe , and ^{58}Fe for excitation energies up to about 4 MeV in the final nuclei, using a lithium beam of 34 MeV. About 20 states are known in this region of excitation for these nuclei, and it was found that only a few of them are excited by this reaction, and with few exceptions these are just the states also excited by (t,p) reactions going to the same final nuclei.

The exceptions are the O^+ states at about 3.5 MeV in each of the final nuclei that are excited by ($^6\text{Li},d$) but not by (t,p). These are very probably the states strongly excited by the (h,n) reaction, and interpreted as two-particle two-hole pairing vibration states in the $Z=28$ proton closed shell.

The group therefore concludes that the ($^6\text{Li},d$) reaction is sensitive to the same neutron and proton pairing correlations that govern the correspond-

ing two-nucleon transfer reactions.

This conclusion supports recent work on the alpha-particle spectroscopic factors, in which the four-particle amplitude is factored into coupled two-nucleon structure factors. The similarity between the ($^6\text{Li},d$) and (t,p) reactions is then explained as the transfer of the proton pair to the ground state and the neutron pair to the ground and excited states as in the (t,p) reactions. The similarity between the ($^6\text{Li},d$) and (h,n) reactions is explained in the same way.

This work suggests further experiments on the two- and four-particle transfer reactions to determine the spectroscopic factors and to relate them to the details of nuclear structure. It also shows that the ($^6\text{Li},d$) reaction can be used to study two-particle excitations that cannot be studied directly by two-particle transfer reactions because the requisite target nucleus is unstable. Such studies should also shed further light on the alpha-particle spectroscopic factors that are important for the study of alpha-clustering in nuclei.

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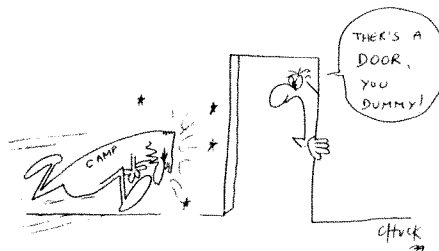
that the altered growth characteristics of transformed cells are dissociable from their altered phenotype (used here to mean low adhesiveness, rounded shape, increased surface microvilli and lack of microfilament bundles). One line of evidence comes from a mutant Swiss 3T3 cell line that has a normal phenotype at 39 °C but which adopts the transformed phenotype at lower temperatures in conjunction with a rapid fall in cyclic AMP levels. Further evidence comes from a mutant Balb/C 3T3 cell line which is defective in the synthesis of N-acetyl glucosamine. It has normal growth properties but a transformed phenotype. When the defect is circumvented by the addition of N-acetyl glucosamine the phenotype reverts to normal. Finally there is evidence that the addition of a major cell surface glycoprotein (CSP or LETS) to transformed cells, which cannot themselves synthesise it, results in a reversion to normal in phenotype without any effect on the growth properties of the cells. Pastan concluded that a reduction in cyclic AMP or cell surface glycoproteins causes the transformed phenotype but that the abnormal growth of transformed cells has a separate and dissociable cause.

Jockusch (Biozentrum, Basle) discussed the possible role of microfilaments in the movement of secretory vesicles from the interior of the cell to the surface. At 25 °C but not at lower temperatures the microfilaments show a preference for attaching to chromaffin granules with the "correct" polarity were they to have the functional role suggested. S. Morris (Max-Planck-Institut, Göttingen) has also used chromaffin granules in his studies of the membrane changes associated with secretion. By freeze fracture he showed that intramembraneous particles (IMP) were not present in areas where inter-granular contacts had been made under the influence of calcium ions. Electron microscopy showed that the sites of granule contact were characterised by a pentilaminar membrane structure.

Whereas Morris's system is but a model for secretion since no plasma membrane is present and no secretion occurs, M. Raff (University College, London) reported comparable results in degranulating mast cells. He showed that the contacts between plasma and granular membranes were characterised by a pentilaminar structure and that these sites neither contained IMP nor could be labelled by ferritin-tagged lectins or antibodies. Since protein displacement was seen before membranes had come into position Raff raised the possibility that the proteins are laterally displaced before fusion and subsequent secretion. That sequence

of events was in part disputed by B. Satir (University of California, Berkeley) whose own observations of mast cell degranulation are consistent with her long series of studies on secretion by ciliated protozoa. In both cases the secretory vesicle has an array of IMP which at least in the protozoal systems, attaches to a complementary array in the plasma membrane. Satir considers that the displacement of the IMP only takes place after fusion has begun and furthermore she has never observed a pentilaminar membrane structure where granular and plasma membranes are in contact.

The release of histamine from the mast cell is triggered by IgE acting by way of the transient opening of a calcium gate (Raff). So is one of the two mechanisms of 5-hydroxytryptamine triggered secretion from the insect salivary gland (M. Berridge, University of Cambridge) whereas the other mechanism uses cyclic AMP as the second messenger. W. Loewenstein (University of Miami) presented evidence that any calcium entering the cell is rapidly sequestered close to its point of entry and would not be able to diffuse into adjoining cells through gap junctions. He also reported experiments, using a series of fluorescently-labelled molecules of known size, which provided data consistent with the idea that the pores in the gap junctions are about 15 Å in diameter, easily large enough to allow the passage of cyclic AMP. That is not to say that cyclic AMP either does or can pass through the pores:



The phenomenon of cell-cell adhesion was dealt with by several contributors. U. Rutishauser (Rockefeller University) has used a novel strategy in looking for a cell surface product that controls the reaggregation of dissociated chick embryo retinal cells. Starting with a heterogeneous antibody to retinal proteins he has found a protein fraction in retinal cell culture supernatants that prevents the inhibitory action of the antibody on cell adhesion. So far a 500-fold purification has yielded an active fraction that still contains three major proteins. The same factor(s) seem to be present on the surface of other solid tissues sug-

gesting that there is no tissue specificity in that component of the process of cell aggregation. M. Steinberg (Princeton University) also believes that "you can go a long way with one kind of glue in tissue morphogenesis". His views are based on the hierarchical sorting out of mixed pairs of any six tissues or of pairs of the same tissue but taken at different stages of embryonic development. The pairs of tissues do not separate on sorting out but one forms a compact core around which the other wraps itself. Steinberg proposes that in every case the core tissue simply has a greater cohesiveness than the wrapper tissue. Since cohesiveness can be roughly equated with surface tension Steinberg has attempted to measure the 'surface tension' of the various tissues by measuring the shape that an aggregate of each tissue adopts under centrifugal force. The results are uniformly consistent with predictions. From all of which Steinberg concludes that tissue morphogenesis can be controlled simply by the inherent cohesiveness of different tissues at different times without the need to invoke specific aggregation factors.

On the other hand L. Glaser (Washington University, St Louis) sticks to the view that there are specific molecular determinants of cell adhesion since he can mimic the cellular adhesion seen with various neuronal cell lines, with their plasma membrane preparations.

The mechanisms underlying the topographically ordered projection of retinal neurones onto the optic tectum of the mid-brain were discussed by S. Roth (Johns Hopkins University). It was R. W. Sperry who first proposed that the projections were determined by specific chemical affinities between each neurone and its site of synapse formation. A more elaborate version of that idea proposes that there are two complementary chemical gradients on retinal and tectal surfaces, one in the anterior-posterior direction, the other in the dorsal-ventral direction. In looking for evidence of the latter gradient Roth has developed a technique in which dorsal retinal cells can be shown to adhere preferentially to ventral tectal halves and *vice versa*. Recently he has found that the ganglioside G_{M2} inhibits this assay and that G_{M2} liposomes behave like dorsal retinal cells in adhering preferentially to ventral tectal halves. He also reported that G_{M1} synthetase, for which G_{M2} is a substrate, is the only enzyme of 30 tested that is more concentrated in the ventral than the dorsal tectum. Although Roth has not yet been able to detect the predicted reverse distribution of G_{M2} in the tectum, it does look as though he

has evidence of an asymmetric distribution of a possible molecular recognition system.

Is this the first toe-hold in putting the process of retino-tectal pattern formation on a firm biochemical footing? Or is it a futile exercise that cannot hope to explain the complexities involved? We all went home! □

Positive ions in the stratosphere

from L. Thomas

OBSERVATIONS of ion composition with rocket and satellite-borne mass spectrometers have made a major contribution to our understanding of the ionised regions of the upper atmosphere but have been restricted to heights above 60 km because of experimental difficulties. In the lower part of the height range the relatively high ambient pressures make it necessary to use a high speed pump for maintaining the spectrometer within its operating pressure range, and the small ion concentrations give low signal currents. Another complication encountered in the rocket observations at these lower heights has been the fragmentation of weakly bound ions through thermodynamic break up at the shock front associated with the supersonic velocity and in collisions, because of the electric fields required to draw ions into the sampling orifice. The difficulties are accentuated at still lower heights because of the greater atmospheric pressures and the increased rocket velocity. Consequently, for stratospheric levels, information is available only from ion mobility measurements but these cannot be used to identify the different ion species present.

F. Arnold, D. Krankowsky and K. H. Marien of the Max-Planck-Institut für Kernphysik, Heidelberg, have developed a quadrupole mass spectrometer with a cryogenically cooled pump, an improved sampling procedure, and a relatively low draw-in potential to reduce the effects of ion fragmentation and, thereby, have managed to extend ion composition measurements down into the stratosphere. They have reported three sets of rocket measurements over the height range 35–55 km (*Nature* **267**, 30; 1977). Although the mass resolution was limited to 2 a.m.u. to ensure sufficient sensitivity, the authors have been able to make a tentative identification of the ions registered. Above

about 40 km the most abundant ions were found to be consistent with proton hydrates, $H^+(H_2O)_n$, with n ranging from 1 to 3. It has been known for more than a decade that such hydrates dominate the positive ion composition over the height range 60–85 km in normal conditions, although some doubt still exists about the appropriate value of n in the ambient ions. Below 40 km, the present results show that a marked change in the predominant ion species occurred within a height range of a few kilometres, and the authors have interpreted the ion masses as resulting from reactions of the proton hydrates with minor neutral species; these species are required to have a higher permanent dipole moment or proton affinity than water to permit the replacement of water molecules or proton transfer to occur. On the basis of aeronautical arguments and supporting laboratory data Arnold *et al.* propose formaldehyde as the neutral species involved. This is known to be a major product of the oxidation of methane, a constituent whose height distribution is reasonably well established. The concentrations of formaldehyde estimated from the interpretation of the ion composition measurements are consistent with those derived in photochemical models (see for example Stewart & Hoffert *J. atmos. Sci.* **32**, 195; 1975). It seems likely that fragmentation has occurred in the ion sampling and, consequently, the particular proton hydrates and formaldehyde-based ions are probably not truly representative of stratospheric conditions. However, as the authors point out, it might be expected that the fragmentation will have removed water and formaldehyde molecules without modifying the central ions.

At stratospheric heights ionisation is produced by the interaction of galactic cosmic rays with atmospheric gases, the primary ions produced being those of molecular oxygen and nitrogen. The formation of proton hydrates from these ions is well understood from laboratory measurements and numerical studies of the ionospheric D region (Ferguson *Rev. Geophys. Space Phys.* **9**, 997; 1971). It would be expected that the ion chemistry of the stratosphere would involve minor neutral species in addition to water, because of the greater variety and concentrations of these species at these heights. In this connection, the inference concerning the upper limit on ammonia concentrations drawn from the absence of relevant ions is of interest. The value quoted is rather smaller than would be expected from loss by photolysis alone (McConnell *J. geophys. Res.* **78**, 7812; 1973) and could indicate the rapid loss of ammonia by collisions with, or con-

version to, aerosol particles.

Considerable interest is currently being shown in the chemistry of the stratosphere. This has arisen out of concern about the catalytic depletion of ozone by chlorine atoms produced by the photodissociation of chlorofluoromethanes (CFMs) transported from the Earth's surface (Rowland & Molina *Rev. Geophys. Space Phys.* **13**, 1; 1975). The observations of ion composition are relevant to the question of whether ion-molecule reactions might play a significant part in the loss of the chlorine-bearing compounds. At the height of the observations reported, the CFMs will be photodissociated and attention needs to be concentrated on the photodissociation products, namely chlorine atoms, chlorine oxide and hydrogen chloride. No exothermic reactions are known to occur between proton hydrates and these three gases (Fehsenfeld *et al. J. geophys. Res.* **81**, 4454; 1976) but the corresponding information relating to the ions identified below 40 km is not available.

Apart from the immediate considerations of environmental protection, the study of the stratosphere has become an active area of atmospheric research. It seems certain that mass spectrometer techniques for the measurement of ion composition will be exploited both in rocket and high-altitude balloon flights. The measurements will serve to complement the observations of neutral minor constituents which are being pursued vigorously and are also likely to inspire further studies of ion-molecule reactions. □

Whither protein structures?

from S. D. Dover

THE pipe-dream of designing and producing a protein to carry out a specific task has been brought nearer reality by the advent of recombinant DNA which offers the possibility of isolating, mutating and cloning particular genes. The resulting change in amino acid sequence specified could produce the intended structural change in the protein. But this will only be possible with an adequate theory of the effect of sequence changes on structure, based firmly on experimental structural information. Without this theory at the level of atomic detail,

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designing new structures would be a hit-or-miss affair.

Interest in the solution of the problem of analysis and, subsequently, prediction of protein structure from sequence has an extensive history. From the studies of Ramachandran (*J. molec. Biol.* **7**, 95; 1963), to those of Levitt (*Nature* **261**, 552; 1976; *J. molec. Biol.* **104**, 59; 1976) theoretically oriented work has been progressing. The basic data for these studies have been the protein structures which regularly appear in the literature. The problem has tended to be approached from the point of view of overall structure with atomic details being left until later.

This has been partly due to the fact that most protein structures available are unrelated. Of the related structures the globins are probably the ones likely to reveal the greatest comparative information. The overall picture of haemoglobin and its allostery has been described by Perutz. Not only is there an abundance of species and within-species mutant haemoglobins of the four-chain type, but also single chain oxygen carriers such as haemerythrin (*Proc. natn. Acad. Sci. U.S.A.* **72**, 2160; 1975), erythrocrucrin (*Eur. J. Biochem.* **19**, 42; 1971) and the monomer/dimer system of the sea lamprey (*J. molec. Biol.* **74**, 331; 1973). Consideration of the chain interactions in atomic detail is likely to be stimulated by recent refinement of sperm whale myoglobin.

Takano (*J. molec. Biol.* **110**, 533, 569; 1977) has just published the first refinement of the deoxy- and met-structures since Kendrew *et al.* (*Nature* **190**, 666; 1961) first interpreted their map. Using new diffractometer data and variants of the well-tried method of alternating phase and realspace refinement (*Acta Cryst.* **A27**, 436; 1971) structures suitable for detailed comparison with haemoglobin were achieved. Takano's stereo diagrams show rather clearly the similarity of structure despite the differences in sequence. The possibility of extensive comparisons of this type is most encouraging, but it does raise a number of problems. Should one compare only whole myoglobin molecules or also separated sections of the chain? How can one objectively make the comparisons between non-identical objects and quantify and display the comparisons?

The relationship between haemoglobin and myoglobin provides information at all levels, but the relative changes are greater than one might want for truly secondary structure. As a starting point movements smaller than those produced by a single amino acid change would be interesting. Such movements would be expected to occur immediately before denaturation, even

though it is a cooperative phenomenon. Stability studies on haemoglobin have already been started (see *Nature* **247**, 341; 1974; *Nature* **255**, 256; 1975). Extension to full crystallographic refinements with crystals at, for example, different pH and ionic strengths might be the key to a number of problems associated with small atomic movements. But will there be PhD students to do all those structures? □

Heat flow at the mantle surface

from Peter J. Smith

SOME time ago Chapman and Pollack (*Earth planet. Sci. Lett.* **28**, 23; 1975) took what they called "a new look" at the Earth's thermal characteristics, producing in the process the first worthwhile map of the global surface heat flow. Then, as now, there were still large areas of the world with few or no data; but Chapman and Pollack had hit upon the idea of filling these gaps with heat flow values predicted from known empirical relationships between heat flow and tectonic age. Both real and predicted observations were then incorporated into a 12th degree spherical harmonic analysis which was translated into a global contour map.

Pollack and Chapman (*Earth planet. Sci. Lett.* **34**, 174; 1977) have now extended their analysis to the much more difficult problem of plotting the heat flow at the crust-mantle boundary. Mantle heat flow is, of course, the total heat flow less that produced or released in the crust; but it is not easy to determine just what the crustal contribution is. Most heat produced in the continental crust comes from the decay of radioactive isotopes whose concentration decreases with depth in some only vaguely understood way. Most heat from the oceanic crust, on the other hand, is released as the lithosphere produced at oceanic ridges cools, although there is also a small but significant radiogenic component.

Combining theory with experimental results, Pollack and Chapman nevertheless manage to estimate these crustal contributions to the total heat flow, whence they derive sufficient data to carry out an 18th degree spherical harmonic analysis. The resulting global contour map of heat flow at the mantle

surface differs from the corresponding crustal surface map in two notable respects. First, a comparison makes it clear that the near equality of continental and oceanic heat flows at the Earth's surface does not extend down to the mantle; at the crust-mantle boundary the mean heat flow in continental regions is only about half of that in oceanic regions (28 mW m⁻² and 57 mW m⁻², respectively). This means that the mantle below the oceans must be considerably hotter than that beneath the continents as several theoretical models have already suggested (see for example, MacDonald *Rev. Geophys. Space Phys.* **1**, 587; 1963).

The second result is that there is much less heat flow contrast within continental regions at the mantle surface than there is at the crustal surface. In Australia, for example, the mean crustal surface heat flow is 81 mW m⁻² in the Proterozoic rocks of the central region and 39 mW m⁻² in the western shield. At the mantle level both regions have heat flows of 27 mW m⁻². Evidently within-continent heat flow variations at the Earth's surface are due entirely, or almost entirely, to regional differences in crustal radioactivity and/or erosion. At the crust-mantle boundary the heat flow contours are simple closed curves centred on the continents and showing no regional complexities.

Finally, Pollack and Chapman are able to demonstrate correlations between the heat flow fields and other geophysical parameters. For example, both total and mantle heat flows correlate positively with the Earth's gravitational potential. However, the mantle heat flow correlates the less positively, suggesting that the broad geographic variations in the gravity field may be due in part to thermally produced heterogeneities in the crust. On the other hand, the fact that the positive correlation remains when the crustal contribution is removed suggests that thermal heterogeneities extend well into the mantle.

By contrast, both the total and mantle heat flows correlate negatively (the latter the more so) with topography, although this is produced by the first-order topography of the Earth (the gross difference in levels between continental and oceanic crust) which is largely a petrological rather than a thermal effect. When the continental and oceanic levels are normalised to remove this petrologically produced contrast, the total and mantle heat flows are found to correlate positively with topography, emphasising, according to Pollack and Chapman, "the significant role that temperature plays in the morphology of both continents and oceans." □

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review article

Economics of alternative energy sources

Martin Ryle*

An important part of the oil and natural gas at present consumed in the UK is used for the heating of buildings, a demand which shows large diurnal, day-to-day and annual fluctuations. The replacement of this energy by nuclear-generated electricity, as at present envisaged, would require the construction of some 250 GW of additional capacity by the end of the century, a programme which does not seem feasible. By incorporating relatively cheap, short term storage in the form of low-grade heat, the generating capacity required to fulfil peak demand could be reduced by more than 50%. As soon as such storage is provided, however, other sources of energy become viable and attractive alternatives, and the UK is well situated to make use of wind, wave, and tidal power. It seems likely that the value of North Sea oil/gas reserves as feedstock to the chemical industry will rise sufficiently to make an early reduction in their consumption as fuel of great economic importance.

It is generally accepted that the energy at present provided by oil and natural gas will be approaching exhaustion by the end of the century, and that the resulting deficit must be made good by the widespread adoption of nuclear power, a large expansion of the coal industry or the development of alternative 'renewable' sources. Each of these possibilities has disadvantages and limitations, and there are problems of development on a short enough time-scale. Because the value of oil and gas as feedstock to the petro-chemical industries (which at present consume about 20% of the total¹) will increase rapidly as the reserves are depleted, it is of the greatest importance to introduce replacements as early as possible; each year gained represents a 4-year extension of supplies of raw material.

Since all of the potentially most useful renewable sources of energy in the UK (solar energy to supply heat or photo-voltaic electricity, wind, wave or tidal power) provide an intermittent or variable supply, their economic viability has always been based on whether they can be justified as 'fuel savers', and it has been assumed that they can never contribute to the 'base-load' in the sense of reducing the total installed capacity of conventional power stations. It then follows that, in order to be cost-effective, the interest on the capital costs of an alternative power system, together with its running costs, must be less than the fuel and running costs of a conventional system, without regard to the capital cost of the latter. Account must, of course, be taken of future inflation, interest rates, and relative costs of fuel in relation to the inflation rate, and the results of such analyses are often unreliable because of uncertainties about these factors, rather than in estimates of the engineering costs.

If the demand for power were constant, this treatment of alternative sources simply as fuel-savers would be a reasonable way of determining the viability of any particular system, but in practice this is far from being the case; there are very large variations in the demand for energy (diurnally, over the periods of 5-10 days typical of UK weather variations, and annually). At present, a com-

paratively small proportion (~ 15%) of the total useful energy is provided by electricity, and the variations in demand for it can normally be covered by the use of pumped hydro-electric stations, gas-turbine generators with a short run-up time, and by arrangements for selling off-peak electricity for water-heating and storage heaters. The fluctuating loads associated with space-heating of buildings and other consumers of oil and natural gas involve peak loads some 3 times the mean energy; at present short-term oil-storage can easily be supplied locally, with longer-term fluctuations covered by depôts or by varying the rate of abstraction through the year. The problem of accommodating the fluctuating loads when these latter energy sources are replaced by electricity does not seem to have been adequately discussed but would, in fact, be acute. I shall seek to demonstrate that a solution of this problem dramatically alters the balance in favour of 'renewable' sources of energy.

In the following sections an estimate is made of the distribution of oil and gas supplies amongst different users; the consequent diurnal, weather-system and annual variations of this additional electricity demand are then discussed in relation to the problems of generation and storage.

The figures are derived from UK Energy Statistics, 1973-75 (ref. 1) two NEDO reports on energy^{2,3}, the conference on 'Energy in the 1980s' organised by the Royal Society⁴, the Building Research Establishment Report on energy consumption in buildings⁵ and other sources. All energy values are expressed in terms of the electrical energy needed to replace them, so that they take account of the efficiency for the consumer of oil- or gas-burning heaters⁶; efficiencies of generation are taken into account in the establishment of the costs of different energy sources.

It is likely that for economic and other reasons the energy consumption in the UK will not increase in the manner assumed in earlier analyses²⁻⁴; better thermal insulation and the economic pressures towards lower temperatures in housing and other buildings, the production of longer-life goods, and better utilisation of industrial waste heat will all tend to maintain the recent trend towards lower energy

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consumption. For this reason it is not unreasonable to adopt the 1972-73 values for the energy consumption, the latest for which a detailed analysis is available³; the relative costs of different generating systems to replace the 1972-73 oil and gas consumption will in any case not be seriously affected even if the total varies from that of 1972-73. Greater uncertainties are caused by the rate at which coal production could (or should) be expanded, the development of more efficient methods of combustion⁶, and the relative costs of manufacturing gas, liquid fuels or other synthetic materials from coal instead of natural oil or gas, especially in relation to fuels for transport.

Although energy data for 1972-73 are used, the capital and operating costs are given in 1975 US \$ prices because much of the most useful data has been presented in this form; an annual inflation rate of 10% has been assumed for 1974-76.

Time distribution of additional requirements

It is assumed that the present (1972-73) UK electricity production (0.77×10^9 GJ p.a.) will continue to supply the present electricity demand, with the exception of the annual contribution (0.24×10^9 GJ) now provided by oil-powered gas turbines. Since the latter are used for peak load boosting, particularly in cold weather, it is assumed that their replacement will require an additional 0.024×10^9 GJ during the summer quarter, 0.06×10^9 GJ during each of the spring and autumn quarters and 0.096×10^9 GJ during the winter quarter; it is assumed that 2/3 of the latter will be needed during the cold spells of the 5-10-day weather pattern.

By taking the present distribution of oil and natural gas between each class of consumer⁷ we can derive the equivalent electrical energy demand and its variation with time of day, with outside temperature, an annually. The conversion efficiency of oil and gas heaters is taken as 60% (ref. 5) and that of electrical heating as 100%, although in some applications, such as water-heating for which off-peak use can help to even out the day-to-night demand, storage is necessary and an efficiency of 95% has been assumed.

The annual variation of the demand for space-heating in domestic and other buildings has been assumed, for simplicity, to be distributed uniformly throughout each quarter in the ratios 50%, 25%, 0% and 25%; this probably gives a rather low figure for the mid-winter requirements.

With present standards of building insulation, night/day switching is essential if a large increase in total demand is to be avoided, and a 50% cycle is assumed. Since the heat loss from a building is roughly proportional to the difference between internal and external temperatures (though it also depends on wind and other factors), there are large fluctuations with the timescale typical of UK weather patterns (5-10 days). A range of winter temperatures of -3 to $+11$ °C is assumed, with average 'whole-house' inside temperatures for the bulk of existing houses of 15.5 °C (60 °F). The hot-water demand is assumed not to vary significantly either annually or with weather conditions, and since 24-h storage is relatively simple it is assumed that two-thirds of the total demand could be restricted to off-peak times.

In the case of Industry it is assumed that, of the total equivalent electrical demand of 0.92×10^9 GJ p.a. at present supplied by oil and gas, there is a constant annual load of 0.72×10^9 GJ, of which 70% is consumed during the day, with an annually varying component of 0.2×10^9 GJ associated with space heating.

Some 95% of the transport energy is associated with road traffic, 67% of which is passenger traffic^{2,5}. Taking a weighted mean conversion efficiency between petrol (14%) and diesel (21%)² and a likely overall efficiency of 60%

for rechargeable batteries, hydrogen fuel cells or other possible storage methods, the equivalent electrical load is computed, with a constant annual rate, but on the assumption that 75% could be restricted to off-peak times in order to assist in reducing the day-to-night fluctuations.

The results are given in Table 1; the magnitude of the total energy demand, 2.1×10^9 GJ, should be compared with the present entire electrical output of 0.77×10^9 GJ, while it is evident that the fluctuations of demand require an extremely large additional installed capacity.

It has been suggested that a major increase in coal production might allow an increase of up to 50% by the end of the century. There are many alternative ways in which this increase might be distributed, but it is clear that part must be used to replace the present oil and natural-gas feedstock to the chemical industry. Assuming that this could be achieved with an efficiency of 90% of that of conversion from oil/gas, this would leave¹ an annual surplus of 0.36×10^9 GJ of equivalent electrical energy at 60% efficiency. This might be distributed (1) as at present, (2) to increase the electricity-generating capacity, (3) to supply direct heat to industry, (4) to increase gas production, (5) to produce synthetic liquid fuels for transport, particularly aviation.

Of these, (5) may be particularly important, and for the present it will be assumed that half the final energy is allocated to this with continuous production, and half to the manufacture of gas. Assuming an 80% conversion efficiency for gas^{4,5} and a similar figure for oil, the total equivalent electrical energy for each is 0.092×10^9 GJ. Since the gas contribution may be reserved, for example for on-peak domestic use, it could reduce the short-term fluctuations in the remaining demand curve, but could only reduce the annual fluctuations if gas stores (such as exhausted North Sea oil wells) or coal reserves and increased conversion plant capacity are provided; any resultant reduction in the annual fluctuations would only be possible, however, if a proportion of houses were equipped with both electrical and gas heating systems. It will therefore be assumed that long term storage is unlikely to be economic, but that a useful reduction in the peak winter loads can be achieved. The effects on the total

Table 1 Demand fluctuations (GW) and annual energy requirements

	Annual energy requirements (10^9 GJ)	Winter				Summer	
		-3°C		$+11^{\circ}\text{C}$		Day	Night
		Day	Night	Day	Night	Day	Night
Domestic:							
Space heating	0.241	49	—	12	—	—	—
Hot water	0.087	1.7	3.3	1.7	3.3	1.7	3.3
Public buildings and so on:							
Space heating	0.233	48	—	12	—	—	—
Hot water	0.038	—	2	—	2	—	2
Industry:							
Plant	0.72	32	14	32	14	32	14
Space heating	0.20	41	—	10	—	—	—
Transport	0.34	5.5	16.5	5.5	16.5	5.5	16.5
Present gas-turbine generators	0.24	36	—	7.5	—	8.7	—
Total:	2.10	213	35.8	80.7	35.8	47.9	35.8
~ 150-h Storage			91			42	
Annual mean					66.5		
Assuming 50% increase in coal production	1.91	187	31	79	31	47	31
~ 150-h Storage			82			39	
Annual mean					60.5		

The intermediate values for the spring and autumn quarters are not tabulated, but are shown in Fig. 1.

residual energy demands are shown at the bottom of Table 1. It can be seen that, while a 50% increase in coal production would make a valuable reduction in the total additional electrical energy needed (besides supplying the chemical industry), large fluctuations on all timescales still remain.

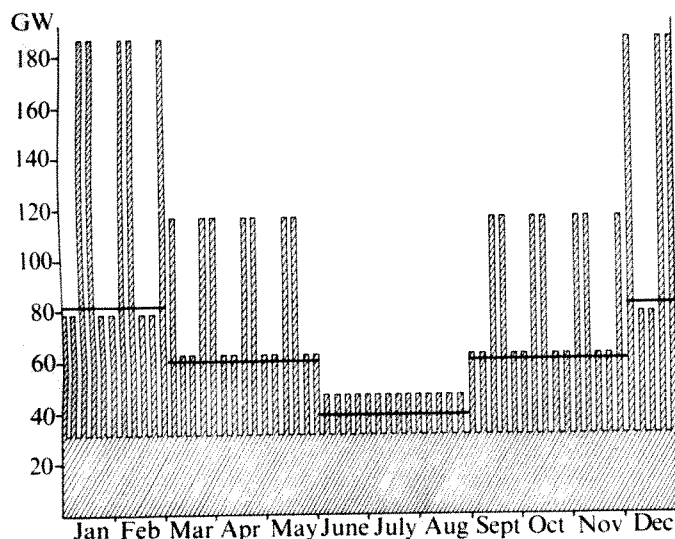
These final figures will be used in the subsequent discussion, and are shown diagrammatically in Fig. 1; it is clear that a very large installed generating capacity would be needed to meet winter peak demands—more than 3 times that needed to supply the mean energy. It is, however, evident that the inclusion of storage can make a dramatic decrease in the installed capacity needed; the provision of ~ 150 -h storage capacity would smooth out both the 24-h fluctuations and those associated with the fluctuations of outside temperature, and permit a 55% reduction in generating capacity.

Central storage systems, whether in the form of pumped hydroelectric installations (for which there are limited further economic or environmentally-acceptable sites⁴), new types of battery, compressed air, hydrogen production or flywheels, all entail a threefold conversion of the energy and are therefore of poor overall efficiency. The major cause of the fluctuations is, however, associated with the provision of low-grade space heating for domestic and other buildings, so the natural solution, both cheap and efficient, is the storage of energy as heat at the point of consumption. The extensive use of heat-pumps could further reduce the peak demand when used in conjunction with such storage, although the efficiency is lowest in cold weather, just when the demand is greatest. The storage of heat over still longer periods has been discussed, but does not seem to be economically feasible^{5,8}.

As soon as storage on the ~ 150 -h time-scale corresponding to that of weather patterns is introduced, however, the main, objection to alternative, 'renewable', sources (that is the variability of output) disappears and it becomes important to examine both the annual variations in the output from such sources, and their capital and running costs relative to those of a nuclear system. Although solar energy has an availability even less appropriate than the constant output of a conventional power station, the outputs of wind- and wave-power systems have maxima during the winter months, in line with the variations of demand.

An attempt is made here to establish the capital and operating costs of both nuclear and alternative systems

Fig. 1 Diagrammatic representation of the fluctuations in demand of the energy at present supplied by oil and natural gas; the timescale of the 24-h and 5–10-day variations has been expanded for clarity. The heavy line indicates the smoothing produced by 150-h storage.



required to supply the demand curve of Fig. 1. Fairly accurate estimates are already available for the costs of solar heating and wind-turbine generators, because both have been investigated and used in other countries for a number of years. Wave-power, tidal and other hydroelectric schemes, and other applications for solar energy will require much detailed study before reliable figures for the energy potential and the capital and running costs become available.

It is assumed that nuclear and wind-turbine generators, and solar heating panels, can all be built with useful lives of 20 years.

A nuclear system

It is difficult to establish the cost of a nuclear power system. A 1975 price for SGHWR power stations based on projections from 1973 figures has recently been given⁹ as \$1,050 per kW of installed capacity, in close agreement with a more reliable contract price (July 1976) of $\$3.2 \times 10^9$ for a 2.4 GW station for New York State¹⁰; the latter figure corresponds to a 1975 price of \$1,130 per kW. This figure does not include the capital costs of the associated fuel re-processing plant, nor of the waste-disposal facilities; details of the latter do not seem yet to have been decided¹¹. The demolition cost of a fossil-fuel station at the end of its useful life is small compared with the capital cost of a replacement, but this may not be true for the demolition of a reactor. It seems unlikely that a value to include the above points will be less than \$1,250 per kW of installed capacity, and this figure will be adopted.

The reliability of nuclear power stations has been discussed by Sorensen¹² on the basis of 1974 international data¹³ and a 1975 survey of US commercial nuclear stations has been made by the Council on Economic Priorities¹⁴. The results represent 'capacity factors' (that is the energy actually generated compared with the maximum possible) of 55% and 60% respectively; the results are worse for the larger stations. While some of the lost capacity was due to routine maintenance, some of which could be restricted to the summer months, it seems unlikely that winter capacity factors $> 70\%$ will be achieved, and this figure will be used.

The true running costs of a nuclear power system cannot be assessed until a full analysis has been made of the problems of transporting fuel, the insurance charges which might have to be covered and the cost, both in manpower and energy, of providing protection for the indefinite future, of the waste-disposal sites.

The cost of energy from UK coal, oil, nuclear and hydro power stations during 1974–75 (ref. 1), (increased by 10% to allow for inflation to mid-1975), was \$3.92, \$6.6, \$1.19 and \$0.30 per GJ respectively, and this is assumed to reflect their total running and fuel costs. In the case of fossil fuel stations, most of it is attributable to fuel costs, but in the case of nuclear stations the subdivision cannot be made until the type of reactor, the corresponding fuel processing costs, and the future effective costs of lower grade ores etc. are known. For the present it will be assumed that the nuclear fuel costs should be divided equally between fuel and running costs. Since some account must be taken of the additional costs mentioned above, a total of \$1.25 per GJ will be assumed, half being related to the installed capacity and half to the energy produced.

Various estimates have been made¹⁵ for the cost and efficiency of energy storage systems, including batteries, compressed air, hydrogen production and flywheels, and range from \$18 to \$45 per kWh; all these systems are, however, intended for reconversion to electrical power. While some central storage may be necessary, the variations in demand are dominated by the low-grade ($< 100^\circ\text{C}$) energy needed for heating buildings, for which cheaper, local, storage may be used. Such a system has recently been installed in the UK at a 1975 price of \$1.5 per kWh (J. Keable, personal communication).

Table 2 The capital and running costs (in 1975 \$) of producing 1 GJ annually with the time variations shown in Fig. 1; for all non-nuclear systems costs include storage

	Capital cost	Annual running cost	Total for 20 years
Nuclear (a) no storage	174	3.6	246
(b) 150-h storage	90	2.1	132
Solar panels	85	0.5	95
Wind	49	0.8	65
Wave	[125]	[3]	[185]

It is now possible to determine the installed capacity, and hence the capital required to provide an annual energy of 1 GJ with the time variations shown in Fig. 1, (1) without storage, (2) with storage at \$2.0 per kWh (allowing for some central storage) (Table 2). The corresponding figures for the running cost per GJ and the total of capital and running costs over the 20-year life of the station are also given; no account has been taken of inflation, since the figures are intended merely to give some idea of the relative importance of capital and operating costs for the different systems.

Alternative energy sources

The first alternative energy source I shall consider is solar energy. The mean annual variation of incident solar radiation (at Kew) is shown in Fig. 2a for (1) a horizontal collector and (2) a vertical collector facing south⁷. About half of the collected energy is due to direct sunlight and half to diffuse light. When used for providing low-temperature heat, simple collectors may be used with typical efficiencies of 40–50% (ref. 5).

This curve is not well matched to the annual variations of Fig. 1, although solar panels are already marginally cost-effective as fuel-savers for pre-heating the supply to hot-water systems⁵. The Building Research Establishment has concluded that about half of the existing houses could each be fitted with 6 m² of panel to provide 7 GJ per annum at a capital cost of ~\$330 or \$47 per GJ; the total annual energy for the number of houses which could be so equipped was estimated as 0.07×10^9 GJ (ref. 5). For new houses, where the cost of part of the roof can be subtracted, or where the design of the house as a whole can include provision for using and storing solar energy, the cost would be appreciably less; more economical designs, both of the panel and, the auxiliary components of the system, are likely to reduce the cost even for existing buildings to ~\$25 for a total annual energy supply of 1 GJ.

In order to contribute the winter values corresponding to 1 GJ having the annual distribution of Fig. 1, the area of panel would have to be increased by a factor of 4.2 (horizontal) or 2.6 (vertical south-facing). Since not all of the houses could be fitted with the latter, an intermediate value of 3 will be assumed. The capital cost of providing 1 GJ having the time-distribution of Fig. 1, must therefore be taken as \$75, although the total annual energy provided is now 0.21×10^9 GJ; the excess energy is not necessarily wasted when 150-h storage is taken into account, since at all times of the year averaging of sunny/cloudy conditions increases the useful energy abstracted, except in summer when the output may be larger than can be used for hot water heating.

Similar installations on public and industrial buildings requiring water heating could increase the total energy to ~ 0.3×10^9 GJ annually, and contribute winter powers equivalent to 0.1×10^9 GJ in the form of Fig. 1. The capital cost per GJ of the latter, including storage, is given in Table 2, together with the small running costs for which a token figure of \$0.5 per GJ is assumed. Other possible developments of solar power are discussed later.

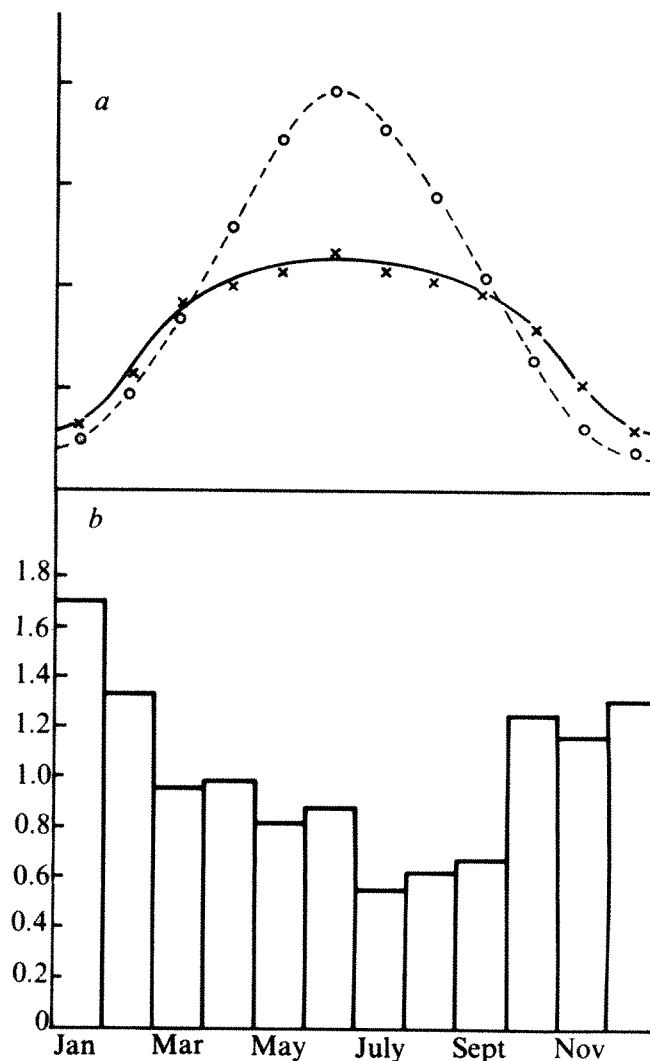
The second possibility is wind power. The annual variation

of the energy available from the wind has been discussed in a paper by Golding and Stodhart¹⁶. The most useful range of wind-speeds (containing some 80% of the total energy), is typically 5–15 m s⁻¹ in the UK and data collected at 7 meteorological stations over periods up to 10 years have been used to derive the useful energy in each month¹⁶. For the five sites situated around the coastal belt and most suitable for wind power, the average annual variation (expressed as a percentage of the mean) is shown in Fig. 2b. The data from two Midland areas with low wind speeds, and one in the Western Isles with very high wind speeds, have been excluded, although in fact the curves are similar.

A comparison of Fig. 2b and Fig. 1 shows that the annual variation of wind power is well matched to that of the demand and, since the variations of wind speed occur with the same 5–10-day periods typical of UK weather, the use of a storage system similar to that described will equally remove these, provided that a more flexible method of load-switching is introduced. Detailed records are available from four of the five sites¹⁶, and have shown that the longest periods of calm (< 4.5 m s⁻¹) vary from 18 to 209 h (the latter over a time span of 10 years); the longest period in the winter quarter with 118 h. Since these calm spells are frequently restricted geographically¹⁷, the actual variations in power available from a distributed wind-power system connected to the grid would be further reduced.

A detailed analysis of the power available annually from

Fig. 2 a, The annual variation of the energy available from solar radiation. ○, Horizontal collector; ×, vertical, south-facing collector. **b**, The annual variation of the energy available from wind power, expressed as a fraction of the mean energy.



a small domestic wind-turbine of given swept area has been made by Rayment¹⁸. Using his figures for the wind at a height of 10 m (corrected for the wind at the height of 35 m appropriate to the large turbines, 55–60 m in diameter, discussed below), the energy available is such as to provide an annual electrical output of $\sim 2.3 \text{ GJ m}^{-2}$ of swept area in the east and southern coastal regions, and 3.2 GJ m^{-2} in the western coastal regions, with values $> 5 \text{ GJ m}^{-2}$ in N.W. Scotland and N. Ireland.

The vertical mixing of the air allows one to site wind-turbines so that their swept area corresponds to between 1.5×10^{-2} and 1.5×10^{-3} of the ground area (refs 19, 20, 21, 22); taking a value of 4×10^{-3} and a mean density of 2.8 GJ m^{-2} , the annual electrical energy would be $\sim 11,200 \text{ GJ km}^{-2}$. These coastal areas have a total area of about $1.5 \times 10^5 \text{ km}^2$.

The whole area could not, of course, be used, though the impact of such wind-turbines, e.g. on agriculture, is minimal. (If 50–60 m diameter turbines ($\sim 1 \text{ MW}$) were used, the structure would be comparable with a 275-kV grid-pylon and their separation would be $\sim 1 \text{ km}$. Since local connections would be at medium-voltage, their visual impact would, in fact, be little different from a nuclear system in which most of the power stations would have to be sited on the coast; the number of (275-kV) pylons within the coastal belt for the latter system would be about two-thirds that for the wind system).

In some areas, as with grid lines, there would be strong environmental objections although, unlike those which apply to a nuclear system, they would be limited to their visual impact. For the present it will be assumed that only one-third of the coastal area considered could be used, with an annual output of $0.56 \times 10^9 \text{ GJ}$. It has been suggested¹⁹ that wind-turbines could also be built in shallow and accessible off-shore areas, such as the Wash and Thames Estuary; while the capital costs of building the turbine and of transmission ashore are greater than those for a land installation, the greater wind speeds would provide an annual energy ~ 5 to 10 GJ m^{-2} instead of the value of 2.8 GJ m^{-2} assumed for the coastal area; the capital costs per GJ may therefore be no greater than for the land installations considered. It seems probable that a further $0.4 \times 10^9 \text{ GJ}$ could readily be provided in this way. The total of $0.96 \times 10^9 \text{ GJ}$ having the annual variation of Fig. 2b would provide an average power of $\sim 43 \text{ GW}$ during the winter quarter, and $\sim 20 \text{ GW}$ during the summer quarter.

Whereas an increase of scale introduces significant economies in conventional power stations, it is not yet clear that this is the case for wind-turbines much greater than 1 MW . The following analysis is therefore based on turbines up to 1 MW for which considerable information on design and cost is now available; for example several installations with capacities up to 200 kW were built in Denmark, and 800-kW and 1-MW turbines were built in France, and were used to provide power for their respective grid-systems during 1955–65^{15,23,24}. The Danish report²³ concluded that by 1975 wind-turbines of this size were cost effective relative to oil-fired power stations even as fuel-savers. The capital cost of their 1957 experimental 200-kW design installed at Gedser, when converted to 1975 prices, was $\$280 \text{ per m}^2$ of swept area for production on a limited scale. A more modern design would have $\sim 40\%$ greater efficiency²³, so this figure, with UK wind speeds, would correspond to about $\$700 \text{ per kW}$ of installed capacity. It is, moreover, likely that a modern wind turbine would be cheaper, largely owing to the development of reliable solid-state devices, which allow the use of a more efficient turbine blade running at fixed pitch (thus removing one of the most serious stress problems) and driving a variable-speed alternator by way of a simple fixed-ratio gearbox, thus avoiding both the complexity of variable-ratio gearboxes and the need for synchronisation with the grid. The output of

such an alternator can easily be varied to present the optimum torque-speed relationship, and would probably be converted to fixed voltage d.c. and transmitted to a sub-station collecting the output from a number of installations for inversion and supply to the grid. A cost significantly below the figure of $\$700 \text{ per kW}$ is therefore to be expected.

The analysis of the Mitre Corporation¹⁵ gives a figure of $\sim \$500 \text{ per kW}$ for a 100-kW installation and $\$300 \text{ per kW}$ for a 1-MW installation—again for a rated wind speed (12 m s^{-1}) appropriate for the UK. Recent design studies²⁵ for 1.5-MW turbines, for lower rated speeds (10.7 m s^{-1}) but rather higher mean wind speeds (8 m s^{-1}) by the General Electric and Kaman Companies in the USA gave costs of $\$449$ and $\$481 \text{ per kW}$ of installed capacity; these use a synchronous alternator which, as discussed above, is not likely to be the most economical system, except for use over a narrow range of operating wind speed. A recent design study by Wesco (G. W-W. Pontin, personal communication) of Redhill for a 46-m diameter turbine and for the assumed UK wind pattern gives a figure of $\$275 \text{ per kW}$.

All the above systems are based on conventional horizontal-axis turbines; it is possible that more economical designs using a vertical axis will be developed^{19,26}.

If the local transmission and substation are included, it is therefore reasonable to adopt a figure of $\sim \$500 \text{ per kW}$ of installed capacity. The remaining transmission costs, as already discussed, are not likely to exceed those of a nuclear system.

From statistics of the wind and an assumption of 90% mechanical serviceability, it is now possible to compute the installed capacity, and hence capital cost, of a wind system needed to provide each GJ with the annual variation of Fig. 1. This figure when added to the storage cost, is included in Table 2.

The Danish report concluded that the running cost of a wind system would be $\$0.93 \text{ per GJ}$, although this figure is probably an overestimate in the light of the improvements and simplification of design discussed above; the corresponding figure from the Wesco data is $\$0.4 \text{ per GJ}$. A value of $\$0.8$ will be used.

The third possibility is wave power. A useful survey of the energy available from wave power has been made by Glendenning and Count²⁷ who give, for the West coast of the British Isles, average annual powers of $50\text{--}100 \text{ kW per m}$ of collector. The medium-term fluctuations in power are very similar to those for overland wind, with a marked variability on a timescale of 5–10 days, while the average available power in winter is again about $2\frac{1}{2}$ times that in summer. Since much of the energy originates in waves generated at distances $> 1,000 \text{ km}$, the 5–10-day fluctuations will not be correlated with those of overland winds, so that a combination of wave and wind power will show smaller fluctuations than either alone.

The cost has been estimated as $\$600\text{--}\$1,200 \text{ per kW}$ of installed capacity²⁷ but this estimate must be regarded as provisional; work supported by the Department of Energy at the University of Edinburgh, the National Engineering Laboratory, and Wavpower Ltd should lead to a more accurate figure after study of the practical problems of mooring such devices in deep water, their survival during storms and the restrictions and dangers to shipping; since some 300 km of collector would be necessary to equal the annual power output of the wind system considered, this latter point is clearly important.

The problems of bringing the power ashore are also much more severe than those involved in either nuclear or wind systems, whether an electrical cable, piped-hydrogen or any other method is used; for the present a capital cost of $\$1,500 \text{ per kW}$ will be assumed, with a serviceability of 80%. The running costs and maintenance of the collector structure will also involve a considerably greater cost per GJ than for a wind system.

Implications for bridging the energy gap

The large fluctuations in the energy demand at present supplied by oil and gas require a generating capacity, for an electrical alternative, some 3 times that needed to supply the mean energy. It does not seem feasible to build the ~266 GW of nuclear generating capacity required before the end of the century. If storage, of ~150 h duration, is included into the system to smooth out the diurnal and weather fluctuations, the installed capacity could be reduced to ~117 GW; the cost of the storage, most of which would be at the point of consumption, would be much smaller than that of the generating capacity saved.

As soon as such storage is introduced, however, a number of the alternative sources of energy can be compared, on an equal basis, with a nuclear system. Table 2 shows that wind energy is the cheapest of all the 'renewable' sources developed so far, and that a system based largely on wind turbines is very much cheaper, both in capital and running costs than a nuclear system; since the figures are based on experience with actual machines comparable in size with those envisaged, any uncertainties in the costs are unlikely to alter this conclusion.

The energy curve produced by the example wind system described is given in Fig. 3, which shows that it would supply half of the demand throughout the year.

The installation of solar panels also provides cheap energy although its poor fit to the annual demand curve increases the effective cost significantly above that of a wind system. It would be useful mainly for hot-water supply, and the installation discussed would contribute the energy curve also shown in Fig. 3. It can be seen that these two sources would together provide a major part of the total demand. The remainder might be obtained either by extending the wind system or by wave power or new types of hydro-electric system²⁸. For the particular case of fuels for transport, plant photosynthesis may become cost-effective. (For example, sugar-beet converts 4.3% of solar energy while methane production from fermentation of sewage, straw and crops such as *Spartina*, which grows on salt-marshes, would not require the use of land suitable for food production⁷).

The actual introduction of the wind system (and solar panels) could be straightforward and quick. An alternative off-peak load-switching system would be needed in place of the rigid time-switches at present in use. The replacement

Fig. 3 The total energy (in relation to the smoothed demand of Fig. 1) provided (a) by the wind system described (shaded), (b) by the installation of solar panels as described, and (c) by both combined.

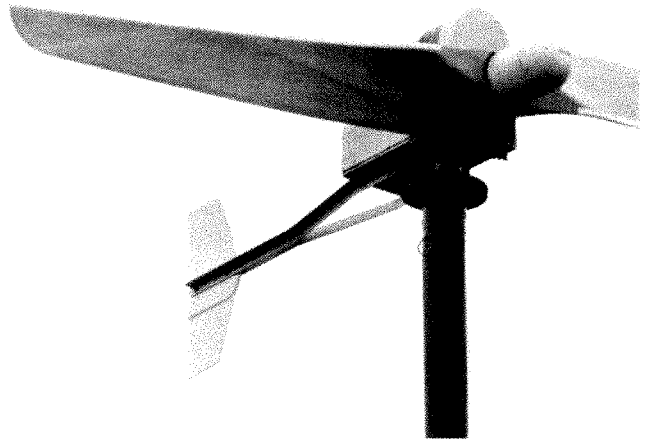
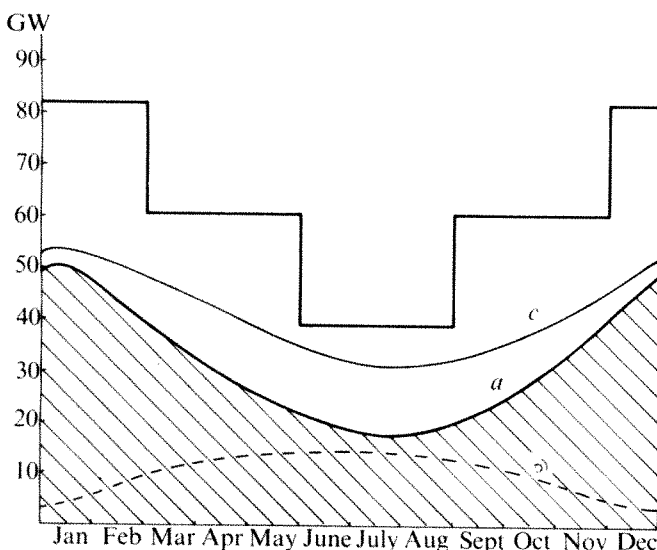


Fig. 4 An experimental 3-m (2-kW) wind-turbine at Cambridge used for the design of efficient blade profiles and methods of load matching and for the investigation of the non-steady loads due to gusts.

of these clocks with a (probably cheaper) detector of a high-frequency carrier transmitted via the mains from the substation (as has been used for street lighting and other switching operations), would allow the load to be adjusted to meet the energy available whatever its time or geographical variations; such a system would also have the advantage of allowing better use of existing generating capacity (for example, weekend surplus, avoidance of high peaks at the start of the off-peak period when thermostat switches are all closed).

As with all alternative systems a single prototype unit of small cost coupled with site surveys allows a reliable estimate of the cost and performance of a complete system, in marked contrast to the expense of building a prototype reactor. In the development both of a prototype wind-turbine, and in subsequent production, about 60% of the cost is accounted for by the tower, gearbox and alternator¹⁵, all of which are virtually 'off-the-shelf' items. The blade design seems to be a problem well within the capabilities of the British aviation industry on a timescale of 6–12 months and a production prototype could be operating within 18 months with the expenditure of perhaps \$1–2 million (the first full-scale Hovercraft flew 8 months after design started).

The subsequent production could build up over a 5-year period to 2,000 units a year (representing, in both cost and tonnage of steel, 10–15% of that of the UK motor industry) and divided approximately equally between the structural steel, the electrical and the aircraft industries. By 1985 wind energy could be providing $>10^7$ GJ annually and the energy consumed in the manufacture of each unit² would be repaid by it in 6–12 months.

In contrast, unless large scale production of an existing reactor design were adopted, the introduction of a nuclear system is inevitably slow. In the case of a new design, such as the proposed FBR (for which there may in any case, be a limitation of ~3% per year in the increase of the system output due to limitations of fuel supply^{11,29}) it would be necessary to build and test, on a timescale of 8–10 years, a prototype before construction of further stations can proceed.

If such a prototype (say, 2–3 GW) were in operation by 1986–87, and a period of perhaps 3–5 years were necessary to repay the energy involved in its construction, fuel extraction and processing and so on, it would only begin to supply useful energy (as Fig. 1) by about 1991, at an annual rate of $\sim 2 \times 10^7$ GJ; the wind system discussed, with a production

of 2,000 units p.a. would, by then, be producing an annual output of about 2×10^8 GJ.

Not only does wind energy seem to be the cheapest way of reducing the energy gap, it is the only way of making a significant contribution within the next 12–15 years, and so allow the preservation of some of the North Sea oil for the future.

Although the discussion has been limited to the specific problem of UK energy supplies, the conclusion may have even greater application in other parts of the world, most of which have either adequate wind or solar resources to support the needs of a lower density of population; the straightforward technology requires only simple maintenance and none of the security problems of nuclear stations. The possibilities of very large export markets should add further incentive to develop the first production version of a 1 MW wind-turbine.

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articles

A chaotic cosmology

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An anisotropic, inhomogeneous cosmological model is proposed in which the inhomogeneity is generated by shear fluctuations. This is a sufficient condition for dissipative heating by collisional neutrinos to explain the present large heat content of the universe, $S_{\text{b.o.}} \sim 10^8$, together with its isotropy and comparative homogeneity on large scales when the photons were last scattered. The model does not require the chaotic motions to be arbitrarily truncated on large scales and isotropises early enough with high entropy to ensure the synthesis of light elements with the observed abundances. A population of black holes which arises in a natural way can also provide the necessary ingredients for a theory of galaxy formation and morphology. The 10^{15} -g black holes, predicted by some authors, are not necessarily expected to be a feature of chaotic cosmologies.

MISNER¹ first pointed out a fundamental problem which measurements of the microwave background isotropy had created². He drew the attention of cosmologists from seeking an explanation for the presence of local enhancements over the background density on galactic scales in terms of random fluctuations to asking why the background universe should display a high degree of isotropy and homogeneity at all. This approach to cosmology was termed 'chaotic cosmology' and in its most general form seeks to show that the observed structure of the universe is demanded rather than merely permitted by the physical processes acting within it. It was thus hoped that

general relativity would predict that the universe is unique in theory as well as in practice, irrespective of its initial state.

Early attempts by Misner to resolve, within the framework of classical relativity, the problems of the contingency of isotropy and the apparent non-intersection of past light cones, together with the fundamental difficulties they encountered at a classical level are well known (see ref. 3). It is still hoped that a complete resolution of the problem will come by appealing to quantum phenomena in the first 10^{-43} s of the Universe's life, but as this route is at present still beset by many conceptual and technical problems⁴ it is interesting to investigate what may be achieved by known classical effects and it is at this level that we address the problem.

The most important unexplained properties of the universe for which cosmologists seek an explanation are probably: (1) The large scale homogeneity and isotropy as indicated directly by the absence of structure in the intensity of the microwave radiation on large angular scales $(\Delta T)/T \leq 3 \times 10^{-3}$ (ref. 2), and indirectly by the observed cosmic mass fractions of helium ~ 0.3 and deuterium $\sim 2 \times 10^{-5}$ predicted by the open homogeneous, isotropic Robertson-Walker (RW) cosmological model⁵. (2) The large heat content of the background radiation, dimensionlessly characterised by the number of black-body photons per baryon, $S_0 \equiv (4a/3k) (T_0^3/n_0) \sim 10^8 \gg 1$ (where a is the radiation constant, k the Boltzmann constant, $T_0 = 2.7$ K, the present radiation temperature and $n_0 \sim 10^{-6} \text{ cm}^{-3}$ the present baryon number density). This is unusually large for a dimensionless physical quantity, but far smaller than the other large cosmic numbers $\sim 10^{40}$ (ref. 6).

The existence of such a uniform physical system in this high entropy state is highly suggestive of there having been a con-

siderable amount of dissipation in the past, although the finite value of the entropy indicates that there could not have existed an arbitrarily chaotic initial state³². (3) The origin of protogalactic inhomogeneities with amplitudes sufficiently large to generate distinct structures on scales $\sim 10^{11}$ – $10^{15} M_{\odot}$ by the present era, yet sufficiently small to avoid a proliferation of compact objects in the same mass range. (4) The absence of developed structure on scales larger than $\sim 10^{15} M_{\odot}$ and the apparent absence of a preferred scale of galaxy clustering over the range 10^{12} – $10^{15} M_{\odot}$ (ref. 7). In conjunction with (3) this presents the problem of explaining the observed spectrum of inhomogeneities at recombination.

The aim of this paper is to outline a chaotic cosmological model in which the above features of the universe might be explained by a hypothesis concerning the type of non-uniformity present at the singularity. There have been many attempts to explain the above problems (1)–(4) in isolation; for example the work of Misner and Matzner⁸ on (1); Rees⁹, Zeldovich¹⁰ and Liang¹¹ on (2). Many workers have discussed aspects (3) and (4) (ref. 12).

The model described here owes much to the chaotic cosmology conceived by Rees⁹, combining other features to improve and extend it.

The Rees cosmology

In brief, the Rees cosmology consists initially of an approximately isotropic distribution of baryons with large amplitude inhomogeneities, $\delta\rho/\rho \sim 1$, on each scale as it enters the horizon. The radiation content of the universe is then generated exothermically by the nonlinear dissipation of the acoustic waves as their scale is encompassed by the Jeans length. For a state of maximal chaos the energy budget is given by $aT^4 \sim \rho_b c^2$, which gives an entropy production–redshift relation, $S = 10^{13} (1+Z)^{-1}$. The shocking of acoustic fluctuations can thus generate the radiation content of the universe by a redshift, $1+Z_f \sim 10^5$. Rees points out that the expected small scale matter clumping on scales less than the photon diffusion length enhances the free–free production rate and enables the created radiation to be adequately thermalised at this redshift. Interestingly, the clumping of matter on scales larger than the photon diffusion length would, in complete contrast, make thermalisation much more difficult than in a smooth model of the same average matter density. This effect could provide an interesting constraint on the existence of lagging cores in the early Universe as envisaged by Harrison¹³.

To prevent the production of more entropy than is observed in the microwave background one must truncate the chaos on the horizon scale corresponding to $1+Z_f \sim 10^5$ encompassing $\sim 10^{17} M_{\odot}$, which is close to the mass of the largest observed structures in the universe. This scheme also explains the approximate coincidence of the epoch of recombination and the onset of matter domination, $Z_{eq} = 10\Omega Z_{rec}$ where Ω is the ratio of the present baryon density to the closure matter density.

Attractive as this conception is, it does however possess a number of undesirable features.

(1) The presence of inhomogeneities having $\delta\rho/\rho \sim 1$ on horizon sized scales with their associated lightlike peculiar velocities is not consistent with the assumption of global isotropy. As a consequence the expansion timescale will accordingly be shortened to correspond with a locally anisotropic (say, Kasner-like) structure for $Z \geq 10^5$. Some of the implications of this situation are mentioned below.

(2) The final entropy, $S_b \sim 10^8$, is principally generated by the dissipation of the largest chaotic mass scales. For shock dissipation the entropy production obeys $S = 3 \times 10^{13} T^{-1}$. This means that during the period $Z \sim 10^9$ – 10^{10} , conducive to light element synthesis, the specific entropy $\sim 10^4$ is too low to produce the observed deuterium abundance $\sim 10^{-5}$, under-producing it by a factor of $\sim 10^8$ and also producing a dis-

cordant helium fraction of 40% in excess of the observed 30%. If one also takes into account the accompanying effects of anisotropy the discord becomes even worse, three anomalous situations being possible according to the degree of anisotropy at the time of nucleosynthesis, $T \sim 10^9$ – 10^{10} K. First, a helium abundance of ~ 60 – 100% in a state of high anisotropy and thermal equilibrium. Second, indeterminate abundances of all light elements if the expansion time scale is less than the weak interaction time but greater than the slowest nuclear reaction time scale. And third, zero abundances of all light elements heavier than hydrogen when the expansion time is less than both weak and nuclear reaction times.

The latter two possibilities can arise in models in which matter motion at near light-like velocities leads to a Lorentz contraction of the expansion time scale in the rest frame of the moving matter^{14,33}.

(3) There is the necessity for an arbitrary cut-off to the chaotic motions on scales larger than $\sim 10^{17} M_{\odot}$. No physical mechanism is suggested which could bring about such a cut-off. The only possible mechanism would seem to be a photon ‘viscosity’ due to the long mean free paths of decoupling photons. The scattering of photons by non-relativistic electrons would give rise to a constant viscosity $\eta = m_e c \rho_e (kT \sigma_{Te})^{-1}$ apparently capable in theory of damping homogeneous shear of infinite wavelength. The recombination of the plasma takes place too rapidly for there to be a viscous period of any significance, however, and any extension of the viscous epoch due to the dissipative heating would seem to be prohibited by the Planckian form of the observed spectrum¹⁵.

(4) The degree of large scale anisotropy compatible with the required inhomogeneity at $Z \sim 10^5$ would almost certainly do violence to the observed constraints $(\Delta T)/T \leq 3 \times 10^{-3}$, on the microwave anisotropy at last scattering unless there occurred sufficient reheating of the cosmic plasma at late times to defer its last scattering until $Z \sim 10$. There are, however, strong limits to the amount of anisotropy energy which can be ‘buried’ in the microwave photons by dissipative processes at late times since distinctive spectral distortions can be expected to arise¹⁶. B. J. T. Jones has pointed out that these distortions cannot yet be predicted straightforwardly as the Kompaneets’s equation describing the evolution of the general photon spectrum is of a different character in a non-uniform environment¹⁶. One would not expect anisotropy to alleviate this distortion in the way suggested by Rees³¹ since the anisotropy must be damped out before the photons are thermalised.

Prolific black hole formation on all mass scales up to $\sim 10^{17} M_{\odot}$ might also be expected and would be in glaring contradiction to observation¹⁷.

(5) The results of Peebles¹⁸ and E. T. P. Liang (preprint, 1976) indicate that difficulties are encountered if one attempts to explain the observed structures on globular cluster and galactic scales as a residue of nonlinear damping. Shocking is highly efficient as an eradicator of large amplitude, short wavelength fluctuations, damping out the adiabatic fluctuations on relevant scales $\sim 5 \times 10^{11} \Omega^{-1} M_{\odot}$ completely and one must appeal to fragmentation of large $\geq 10^{14} M_{\odot}$ clumps or isothermal perturbations to describe the presence of galaxies. In fact the damping is probably much more efficient and even larger scales removed than those predicted by Liang since his calculation based upon a simple wave picture in which the self-interaction of single sound waves is adequately described but their mutual interaction is not. A more complete picture of ‘acoustic turbulence’ including these effects might give rise to more efficient dissipation over larger scales than the simple wave picture.

A new model

We describe here an initially anisotropic and inhomogeneous cosmology which generates the observed microwave radiation by the dissipation of chaotic motions but in which the chaos is

damped out on all scales at early times. In this way one enables light element synthesis to proceed as in the standard RW models and the radiation background to become isotropic with Planckian spectrum without having to invoke small scale clumping or re-ionization at late times.

We shall take the singularity structure to be defined by an anisotropic and inhomogeneous zero entropy state filled with pressureless dust. This is locally equivalent to a Kasner universe with large amplitude, $\delta\rho/\rho \sim 1$ matter perturbations on all scales when they enter the horizon. The work of Belinskii *et al.*¹⁹ indicates that such a representation is sufficiently general to constitute an open neighbourhood in the superspace of all cosmological solutions to the Einstein equations. It is also a self-consistent picture since the presence of global spatial inhomogeneity implies anisotropy in the expansion dynamics. We now hypothesise that the inhomogeneity be limited to that class which is generated by equilibrium shear fluctuations, the meaning of and reason for this restriction will be explained below.

The very early evolution of this model is envisaged as proceeding in similar manner to the Rees cosmology, entropy being generated by relativistic shock damping of the large amplitude acoustic waves, leading to a growth of entropy according to the relation $S = 3 \times 10^{13} T^{-1}$, as above. This process proceeds efficiently since the relatively soft equation of state guarantees that the local sound speed is much less than the peculiar velocities induced by the inhomogeneity. More rigorously, solution of the fluid dynamical equations for test motion in the anisotropic background shows that the velocity field satisfies

$$U_k = f[(x_k \pm ((ct^{1-p_k})/1 - p_k)]$$

where U_k is the fluid three velocity, f an arbitrary differentiable scalar function and p_k are the usual Kasner indices satisfying $-\frac{1}{3} \leq p_1 \leq 0 \leq p_2 \leq \frac{2}{3} \leq p_3 \leq 1$; flow singularities thus develop.

This process will continue to produce entropy until the temperature falls to $\sim 10^{11}$ K when the influence of weakly interacting particles^{8,20} becomes crucial to a discussion of the dynamics. It is at this temperature that the viscous action of decoupling electron and muon neutrinos can lead to the damping of the expansion anisotropy. We consider the equilibrium neutrino case, with the neutrino-electron scattering time shorter than the expansion time, $t_{\nu e} < t_{\text{exp}}$, in which the corresponding coefficient of viscosity is derived from the Feynmann Gell-Mann theory of weak interactions, $(20)^*$, with $\eta \sim \rho t_{\nu e} \sim T^{-1}$. (The existence of neutral currents make a negligible difference to the result.) We describe the anisotropy by the ratio of the anisotropy energy density, to that of the thermal energy, defining

$$A \equiv \rho_B/\rho_T = \sigma^2(NaT^4)^{-1}$$

where σ is the kinematic shear and N the weighting function for the particle species. It can be shown that the equilibrium neutrino assumption restricts the allowed anisotropy to $A \sim 10^6$. (This is demonstrated by comparing the ratio of the neutrino-electron collision time, $t_{\nu e} \sim 10^{59} T^{-5}$ to the expansion time $\sim (G\sigma^2)^{-1/2}$ at the dissipation epoch when $T \sim 5 \times 10^{10}$ K). By calculations analogous to those of Misner²⁵, it can be shown that in this case the viscous action of the neutrinos is always effective in reducing the anisotropy energy from its maximum allowed equilibrium value, $A \sim 10^6$, to $A < 1$ within an expansion time and the growth of entropy is $S/S_i \sim 10^5$, where S_i is the value of the specific entropy before the neutrino viscosity became effective. This is precisely the amplification factor necessary to augment the shock generated entropy, $S_i \sim 10^3$, to the required value, $S_0 \sim 10^8$ for the products of nucleosynthesis at $T \sim 10^9$ K to be in agreement with observation. (For a general temperature dependent viscosity $\eta \sim T^n$, we find

$$S/S_i \sim 5 \times 10^{6(1-n)/(2-n)} / T_i^{(n^2-1)/(2-n)}$$

where T_i is the adiabatic temperature at which viscous dissipation commences, so $\rho_{vi} = NaT_i$. These results can be obtained from those of Matzner³⁴.) Some vorticity will also be created in the shock fronts and by the viscous dissipation itself²¹ but this is created only on $\lambda < vt$ scales where photon viscosity will subsequently damp it out and it thus has questionable relevance to turbulent models of galaxy formation.

Now we also notice that since the inhomogeneity is generated by shear fluctuations it will also be damped out by the neutrino viscosity, even on scales larger than the horizon. No acausal process is implied here, for as the infinite wavelength homogeneous shear is damped so is its global fluctuation. More formally we know that a space which is locally isotropic everywhere must be spatially homogeneous and here it is the action of the constraint equations of elliptic character that force the space into a homogeneous state. Such an effect has been found in the case of small inhomogeneity by Liang²² but was not interpreted physically and it is in fact also apparent in the results of Stormer²³. The smoothing process can also be viewed in terms of the initial value problem. The evolution equations are hyperbolic but the initial conditions are set so that non-communicating domains share some common features. In particular, fluctuations on scales smaller than the horizon are everywhere damped and hence appear damped on scales greater than the horizon (M. A. H. MacCallum, personal communication).

Thus the viscous mechanism is also effective in removing the inhomogeneity on all scales by the time the temperature has fallen to $T \sim 10^9$ K and on large $\lambda > ct$ scales the universe is then effectively the standard Robertson-Walker model—the structure on smaller scales is discussed below. The model can then generate the observed helium and deuterium abundances in the usual way with the proviso that the entropy dependence of the latter is modified by the effects of its small scale fluctuation.

Finally, we turn to the problem of the possible origin of suitable protogalactic structures. Clearly we could make the *ad hoc* assumption that there exist growing curvature fluctuations with $\delta\rho \sim 10^{-6}\rho$ on all scales when they enter the horizon as required in the theories of Zeldovich *et al.*²⁶ and Gott and Rees²⁷, which would escape any viscous dissipation on scales greater than the horizon. Such a state of affairs is unconvincing, however, and a second possibility arising naturally in the model is more interesting.

Meszaros²⁴ has proposed a theory of galaxy formation based on the hypothesis that a fraction of the matter in the early Universe collapsed to form a population of low mass black holes, the subsequent fluctuation in their number density constituting a non-decaying isothermal density perturbation. The absence of electromagnetic coupling between the black holes and the background radiation field ensures that they behave as entropy perturbations experiencing no damping by radiative pressure. Their amplitudes on scales smaller than the horizon remain frozen until the radiation ceases to dominate the expansion dynamics, after which linear growth proceeds in the usual way. This scenario is shown to lead to a consistent theory of galaxy formation and morphology at later times. In Meszaros' work the origin, number density and mass of the black holes are all arbitrary parameters chosen as initial conditions to guarantee the right amplitudes at recombination. In the model described above, however, such a black hole population arises naturally during the viscous damping period while the model is undergoing isotropisation. The shocking of inhomogeneities when they enter the Jeans length will not be completely efficient and when the sound speed is c_s we would expect some inhomogeneities of amplitude δ to collapse into black holes with a probability $\sim \delta \exp(-\frac{1}{2}c_s^2\delta^{-2})$ (ref. 17), if their amplitudes are appreciable on scales intermediate between the Jeans and horizon masses. At first sight it might seem from the results of Carr that black holes will form prolifically on all scales below $\sim 1M_\odot$ leading to $\rho_{bh} \sim \rho_{\text{dust}}$ in this mass range and thus causing the Hawking evaporation¹⁷, at the present epoch of

those with $M \sim 10^{15}$ g to violate the limits on the observed γ -ray flux in the 100 MeV range which requires $\rho_{bh} (10^{15} \text{ g}) < 10^{-6} \rho_{dust}$. We claim, however, that black-hole collapse will only occur on horizon sized scales during the isotropisation and homogenisation process itself when $A \leq 1$. This will produce a population of small black holes with mass $\sim 1 - 10^4 M_\odot$ and their density must satisfy $\rho_{bh} \leq \rho_{dust}$. The reason for this restriction in the mass range is that the calculations of Carr model the inhomogeneity as a curvature fluctuation by joining different RW models together. The space-time is thus allowed global isotropy although here global anisotropy is required for self-consistency. Now, whereas in an isotropic universe the collapse time for the matter $\tau_c \sim \rho_\gamma^{-1}$ equals the expansion time τ_{ex} and collapse can readily occur, this will not be the case in an anisotropic background where the expansion rate is

$$\left(\frac{\rho_B}{\rho_\gamma} \right)^{\frac{1}{2}}$$

times faster.

Thus, as the collapse time remains $\sim \rho_\gamma^{-1}$, anisotropic dynamics guarantee $\tau_c \gg \tau_{ex}$ and there is insufficient time for the black holes to form. A more physical explanation is that the dominant anisotropy pressure determines the Jean's mass, making it equal to the horizon size. Since the gravitational collapse takes place on scales larger than the Jean's mass, overdense regions collapse while still larger than the horizon size to form separate universes¹⁷, and not black holes in our Universe. (This problem is now being investigated in detail with B. J. Carr.) This argument indicates that one might not expect to observe the Hawking effect today even if the early Universe were highly chaotic, unless 10^{15} -g black holes were present in the universe *ab initio*.

By generalising the calculations of ref. 24 it can be shown that if the number fluctuations in the black hole distribution satisfy

$$\frac{\delta N}{N} = N^{-n}$$

the perturbations will form discrete bound systems of mass M at a redshift

$$1 + z_b \sim 4 \times 10^{6-11n} \Omega^{3/4} t_i^{-1} \left(\frac{b}{1 M_\odot} \right)^n \left(\frac{M}{10^{11} M_\odot} \right)^{2/3-n} \quad (1)$$

where b is the initial mass of the hole and t_i the formation time. But, we know $b(t_i)$ in the isotropising model from the expression for the horizon size³⁵, $b \sim 10^4 t_i M_\odot$.

For an initially Poisson fluctuation, $n = \frac{1}{2}$, there is a minimum of parameters in equation (1) and the largest scales condense out first; where for partially correlated fluctuations, $n = 2/3$, all scales condense simultaneously. In both cases we can have $1 + z_b < 10$ for galaxy and cluster masses. This scheme of protogalaxy formation has a number of interesting points in its favour since the perturbations are not damped by photon diffusion in the radiation era, a process which smooths the interesting $\leq 10^{13} M_\odot$ scales from the spectrum of adiabatic fluctuations. Also, from equation (1) we see that there is a continuous scale of galaxy clustering and the existence of the galactic scale as a lower bound arises because gas dynamic processes are able to destroy the clustering on smaller scales. It is supposed that these non-gravitational effects imprint the characteristic scales and structure on galaxies rather than the precise spectral form of the density inhomogeneities³⁰.

There seems to be no observational evidence against a large number of small black holes having, say, the same mass spectrum and distribution as stars²⁴. The model might be open to observational test by the effect pointed out by Dahlbacka *et al.*²⁸. If gas which falls into a black hole cannot find a more efficient mechanism than electron ion collisions to maintain $T_e = T_{ion}$, (for example through turbulent or magnetic effects),

then a feature could be expected in the γ -ray background in the 10–20-MeV range.

Conclusions

I have tried to show that a partially chaotic model of the early universe can provide an explanation for the entropy and uniformity observed today together with a viable scheme of protogalaxy formation and clustering. The model has the following input. (1) The inhomogeneity is restricted to that class generated by shear fluctuations on all scales at the singularity. And (2), There is neutrino equilibrium, such that $t_{ve} < t_e$ which requires that the anisotropy, as measured by

$$A \equiv \left(\frac{\rho_B}{\rho_\gamma} \right)^{\frac{1}{2}}$$

is bounded above.

The restrictions (1) and (2) in conjunction describe 'equilibrium shear fluctuations'. Both these restrictions have phenomenological motivation. First, we know that the isotropy and finite entropy observed today limit the initial degree of chaos to a finite level³², and second, if there did not exist thermal equilibrium of the neutrino mediated weak interactions when $T \sim 10^{10}$ K then the synthesised abundances of helium and deuterium would be either indeterminate or non-existent¹⁴. The degree of generality admitted by the assumption (1) is difficult to assess precisely since no good criteria exist to determine physical cardinality. But as the influence of shear fluctuations becomes infinite as the singularity is approached whilst that of curvature fluctuations becomes negligible, the restriction (1) may in some ways be natural for a chaotic model.

Finally, two important and as yet undiscussed problems still loom large on the horizon of chaotic cosmology at a classical and a quantum level. We could ask why the discussion should be limited to baryon symmetric models of the initial state or indeed is the whole concept of a zero entropy (non-thermal) yet chaotic initial state a contradiction in terms in any case?

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A physical interpretation of the Haicheng earthquake prediction

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A possible explanation for the successful prediction of the 1975 Haicheng earthquake is that the earthquake was triggered by a deformation front that propagated 1,000 km through NE China at a velocity of about 110 km yr⁻¹. The various phenomena that were used to predict the earthquake can be explained by the deformation front.

ON 4 February 1975, an earthquake of magnitude 7.3 occurred near Haicheng in Liaoning Province, north-east China. The earthquake had been accurately predicted by the Chinese, and thus was the first major earthquake to have been successfully predicted.

The prediction of the Haicheng earthquake took place in four stages—long-range, mid-range, short-range and imminent prediction^{1,2}—each stage representing a narrowing of the prediction window in space and time. Each stage was based on various observations judged to be precursory to an earthquake.

In 1970 a national meeting was held to discuss regions of particular seismic hazard within the People's Republic of China. That meeting followed the occurrence of a series of strong earthquakes that had begun with the two Hsingai earthquakes of 1966 and had culminated with a magnitude 7.4 even in the Pohai Gulf in 1969 (Fig. 1). It was postulated that these large earthquakes were migrating to the north-east, and as a result the area north of the Pohai Gulf, including Liaoning Province, was designated as a region in which a large earthquake, of magnitude 5 to 6, was to be expected in the future. The Jinzhou, Zhuanghe and Yalu River faults, all prominent NNE striking faults in southern Liaoning, were apparently recognised as the major structures along the NE trend of earthquake migration and were singled out for special attention. The result of this long-range prediction was an increase in scientific investigation in Liaoning Province, in particular, levelling along the Pohai coast, installation of new seismic stations and initiation of short level lines across the above mentioned faults.

In June 1974 another meeting was held to review the data collected as a result of the intensified studies made in the preceding 4 yr. At that meeting the prediction time span was shortened, to predict an event of magnitude 5–6 within 1–2 yr in the northern part of the Pohai Gulf. This was based on three main observations: (1) microearthquake activity had increased several-fold, beginning in late 1973, over the entire Liaoning Province. These were not foreshocks, being too widespread. This increase in seismicity was reported for the area enclosed by Shenyang, Tantung and Dairen, a triangular region 200 km on a side. (2) Levelling showed that the greatest gradients in vertical motion for the entire Pohai region occur in the Liaotung Peninsula. (3) Short level lines across the Jinzhou fault at Chin Hsien showed rapid vertical movements beginning in mid to late 1973.

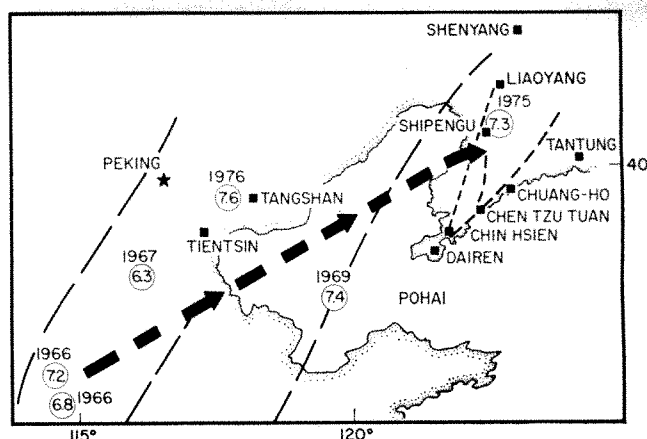
The result of this prediction was to further intensify the scientific efforts, to begin a mass education programme to warn the general populace of the danger of a forthcoming earthquake and to begin organising amateur groups to report anomalous phenomena.

In mid-December 1974, a swarm of earthquakes occurred in the vicinity of Liaoyang, about 70 km north of the epicentre of the Haicheng earthquake. The principal shock was of magnitude 4.8 and was widely felt. This activity is unusual in this region of normally low seismicity, and following these earthquakes the Revolutionary Committee of Liaoning Province issued alerts concerning the possibility of a large future earthquake. Reports of anomalous behaviour of animals and water wells, which had increased before the Liaoyang earthquakes, continued to increase in the region Shenyang–Tantung–Dairen. In mid-January 1975, it was predicted that an earthquake of magnitude 5.5–6 would occur within this region in the first half of 1975. This further shortening of the time span of the prediction (but not of the span in space) seems to have been in response to the Liaoyang earthquakes and the increase in amateur observations of anomalies.

The foreshocks of the Haicheng earthquake began on 1 February, and peaked in the evening of 3 February. Ten of these events were greater than $M=3$, two greater than $M=4$, and were widely felt in the epicentral region. Also beginning on 1 February, observations of anomalous animal behaviour and water-well changes began to migrate to the vicinity of Haicheng and Yinkow (the epicentral region), a tilt anomaly at Shenyang began, and many anomalies in electrical resistivity and radon emanation were observed. These observations, particularly the foreshocks, led to narrowing the prediction area to the Haicheng–Yinkow area on the day of earthquake, and emergency measures were carried out as the earthquake was predicted to occur within 1–2 d.

The earthquake occurred on the evening of 4 February 1975. It was a left-lateral strike-slip event, on a north-west striking fault plane with an aftershock zone about 70 km

Fig. 1 Map of NE China, showing all earthquakes larger than $M=6$ since 1966, the localities mentioned in the text and the path of the deformation front.



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long³. This fault plane had evidently not been previously recognised, and it has been suggested that it is a newly formed fault⁴.

Discussion

I have attempted briefly to sketch the observations and events that occurred before the earthquake. In one respect or another, most of the observed phenomena can be considered anomalous; what remains to be determined is which of these phenomena were truly precursory to the earthquake. That is, which phenomena had an actual physical connection with the earthquake and could therefore be used reliably to infer the coming of the earthquake.

When I first considered these problems I found that I could, based on conventional seismological thinking, readily dismiss as irrelevant virtually every allegedly precursory phenomenon save the foreshocks. Yet the prediction was made, and accurately, before the foreshocks. That this could have been done based on totally irrelevant observations seems very unlikely, since this would imply that it was fortuitous. But Liaoning Province is an area of normally low seismicity—it seems that in the 3,000 yr of Chinese history, the Haicheng earthquake is by far the largest earthquake ever to have occurred in Liaoning, only two earthquakes greater than magnitude 6 having occurred there in 3,000 yr. It is probably this fact that led the Chinese consistently to underestimate the size of the Haicheng earthquake: at all times they expected it to be less than magnitude 6. Thus barring the possibility of chance, I am forced to consider some rather unconventional possibilities to explain some of the forerunning phenomena.

Among the most interesting of the phenomena is the north-easterly migration of epicentres. That this migration occurred is beyond doubt, but what is important is whether or not there is some causative process responsible for the migration that would allow the Haicheng earthquake to be predicted by induction. Incidentally, the Chinese did not use pure induction. If they had, they would have predicted an earthquake of magnitude 6.5 to 7.5, the size of the other earthquakes in the series, and thereby would have correctly predicted the magnitude of the Haicheng earthquake. Instead, probably for the rather conservative reasons given above, they underestimated its magnitude.

Such migrations are known^{5,6}. One possible explanation for them might be that the change in the strain field produced by one earthquake triggers, with some time delay, the next shock. One cannot explain the north China migration so readily, however. The distance between the successive earthquakes is considerably larger than the fault lengths that can be associated with them, so that the strain change in one epicentral region resulting from the previous earthquakes in the series will be very small. Furthermore, the successive earthquakes did not occur on the same fault.

Two possibilities remain—either the migration was illusory or it occurred as a result of some migrating stress source of presumably deep-seated origin. In what follows, I refer to such a moving stress source as a 'deformation front'.

The second most interesting phenomenon was the strong increase in microearthquake activity that began in late 1973. This increase occurred over the entire province, so it could not have been caused directly by some process in the focal zone of the Haicheng earthquake, nor was it an artefact of increased instrumental coverage.

We are drawn, then, to suspect that if there is some relationship between the increase of microearthquake activity and the ensuing Haicheng earthquake, that it must have been caused by a rapid regional increase of stress level over all of Liaoning Province that later resulted as one of its manifestations in the Haicheng earthquake. This of course is not inconsistent with the earlier suggestion made to explain the earthquake migration, and that late

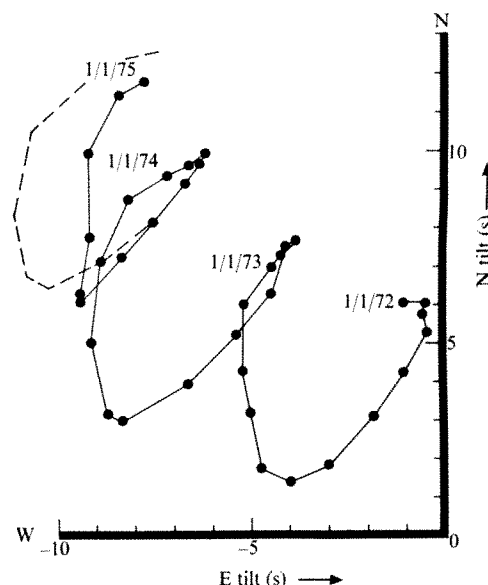


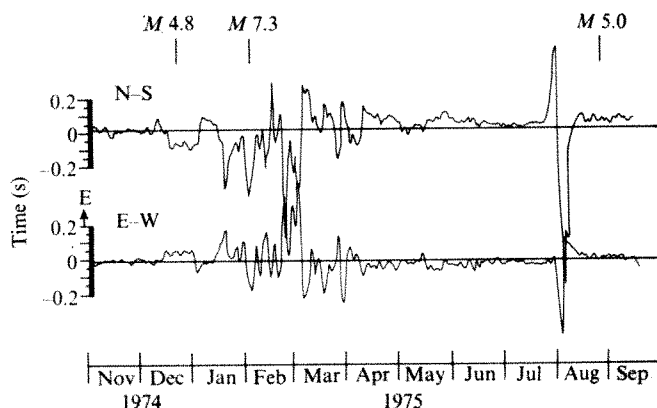
Fig. 2 Monthly tilt recorded at Shipengu. Recorded tilt is given by solid lines and dots. The average annual tilt cycle for 1972 and 1973 is shown as a dashed trace in 1974.

1973 marked the arrival of the stress source in Liaoning Province.

Regional levelling showed that vertical movements had been occurring at a steady rate before 1971 (ref. 2). Short baseline levelling repeated daily at Chin Hsien, however, showed an abrupt onset of tilt, down to the north-west, beginning in mid to late 1973, a time that coincides with the increase in seismicity. This levelling line crosses the Jinzhou fault, but no discontinuity in vertical movements occurred at the fault so that it apparently represents regional deformation. Levelling at Chuang-ho also is consistent with anomalously rapid vertical movements in the period 1970–74, but that at Cheng Tzu Tuan is inconsistent with this. Following the onset of tilt at Chin Hsien, a long-term tilt anomaly was observed with tiltmeters at Shipengu, beginning in June 1974, 8 months before the earthquake (Fig. 2).

Daily tilt rates at Shenyang, farther to the north, are shown in Fig. 3. A short-term anomaly was recorded with these instruments for 4 d before the earthquake. That anomaly was probably not due to deformation within the epicentral region, however, because no such anomaly was

Fig. 3 Daily tilt rates at Shenyang. The baseline assumed for integration is given by the horizontal lines. The integration was only carried to July because of the large tilt anomaly at the beginning of August, which was apparently caused by heavy rainfall.



observed at Shipengu, only 20 km from the epicentre, and no coseismic tilt was recorded at Shenyang. We notice a long-term tilt anomaly at Shenyang, however, marked by the very high tilt rates beginning in mid-December 1974, and lasting until April or May of 1975.

The three tilt anomalies are compared in Fig. 4, where the magnitude of the tilt vector is plotted against time, and the time scales are shifted so that the onset of the anomalies coincide. The time scales in Fig. 4 for Shipengu and Shenyang were expanded by factors of 3.8 and 5.7, respectively, with respect to Chin Hsien, and the magnitude reduced by a factor of four at Shenyang. The anomaly at Shipengu was taken as the difference of tilt recorded in 1974 from the average annual cycle of the previous 2 yr, indicated as the dashed trace in Fig. 2. The Shenyang anomaly was obtained by taking 10-d averages from the data shown in Fig. 3 and integrating them. A steady north-west drift was assumed to be the secular trend there, and was used as the baseline for integration. This procedure will result in the Shenyang tilt anomaly being contaminated with an unknown annual term, but the very good correlation with tilt at Chin Hsien (Fig. 4) shows that the anomaly is not masked. The details of the correlation are lost at Shipengu, where we have only monthly averages, but the overall shape of the anomaly there is also very similar to the others.

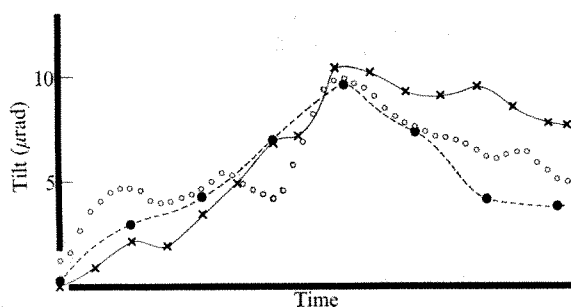


Fig. 4 Comparison of the tilt anomalies at three sites. The time scales at Shipengu (●) and Shenyang (×) were expanded by factors of 3.8 and 5.7, respectively, to that at Chin Hsien (○). The magnitude of the tilt vector is plotted. The magnitude of the anomaly at Shenyang has been reduced by a factor of 4.

The strong correlation of these three anomalies suggests that they are measures of the same phenomenon, a rapid regional deformation that propagated through Liaoning Province in a north-easterly direction.

Returning to the original hypothesis, if we assume that a deformation front propagated through China on a path indicated as the heavy dashed line in Fig. 1, we can show the sequence of events as a space-time diagram projected along that line (Fig. 5). There we can see that a deformation front moving at a velocity of about 110 km yr^{-1} can account for the increase of seismicity in Liaoning Province, the successive onset of tilt anomalies at Chin Hsien, Shipengu and Shenyang, and the triggering of the Liaoyang swarm and the Haicheng earthquake.

The details of the events in Liaoning Province are shown in a similar space-time plot in Fig. 6, which shows the magnitude of the tilt anomalies and, as arrows, the direction of tilt at several times during the passage of the deformation front. Initially, tilt was NNW at Chin Hsien, SSE at Shenyang and east at Shipengu. As the deformation front passed, all tilts rotated clockwise to the west. The Liaoyang swarm occurred just as the maxima arrived at that point, but the Haicheng earthquake occurred after the maxima had passed.

The compressed time scales at Shipengu and Shenyang relative to Chin Hsien indicate that the direction of motion

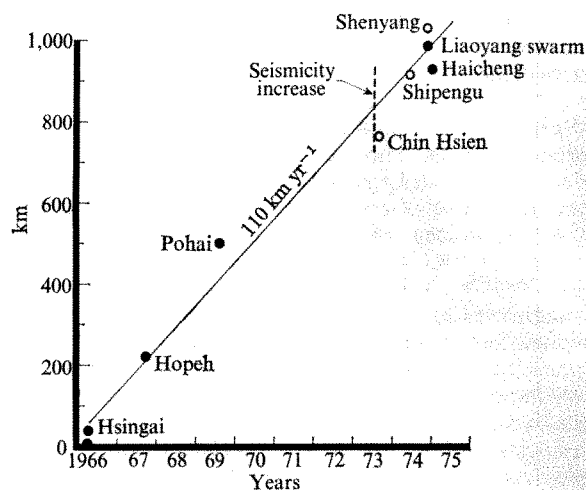


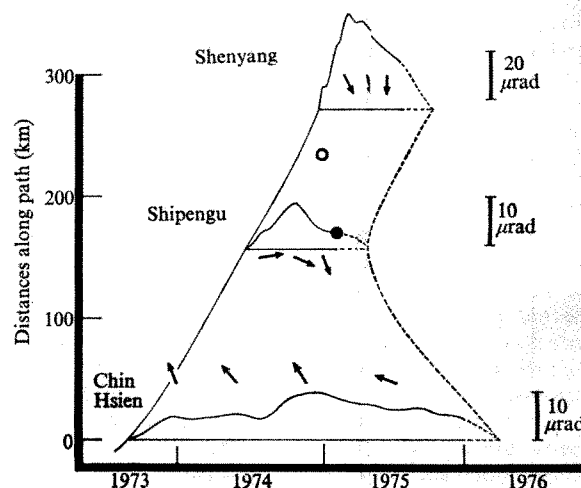
Fig. 5 A space-time diagram projected on a $N60^\circ E$ line.

of the deformation front turned to the east as it entered Liaoning Province in a way suggested in Fig. 1. This rotation was about an axis south of Chin Hsien, so that the propagation velocity of the front was greater by a factor of 3.8 at Shipengu and 5.7 at Shenyang than that at Chin Hsien. This also explains why the anomalies at Shipengu and Shenyang began after, but ended before, the anomaly at Chin Hsien and why the apparent velocity between these sites (200 km yr^{-1}) was greater than velocity deduced from the earthquake migration. Note also that the width of the deformation front was at least 300 km, and that this would include all the earthquake epicentres along the propagation path (Fig. 1).

Two other anomalous phenomena may also have been caused by the deformation front: a rapid rise in sea level of about 1 cm recorded by tide gauges on the Liaoning coast late in 1973, and an increase in magnetic field intensity in Liaoning relative to Peking that began in late 1973 or early 1974 and lasted until late 1975 (refs 1, 2).

There are only two inconsistencies, the lack of increased tilt at Chen Tzu Tuan, in the period 1970–74, and the 1976 Tangshan earthquake, which according to this model should have occurred late in 1968. The first can be explained if the levelling was done early in 1974 at Chen Tzu Tuan.

Fig. 6 Space-time diagram for Liaoning projected on a $N60^\circ E$ line. The magnitude of the tilt anomalies are shown against time, and the direction of the tilt vector indicated by arrows for several periods of time during the anomalies (up is north). The Liaoyang swarm is indicated by an open circle and the Haicheng earthquake by a closed circle.



With regard to the Tangshan earthquake, it should be pointed out that there are two conditions necessary for an earthquake to be triggered by a deformation front. The first is that a zone of high stress, in which an earthquake is already nearly imminent, lies within the path of the deformation front. The second is that the additional stress produced by the deformation front be additive (tensorially) to the pre-existing tectonic stress. The second condition may not have been met at Tangshan.

Conclusions

Although the Chinese did not use the anomalies at Shipengu and Shenyang in their prediction, I have shown that four of the observations upon which they based their predictions—the migration of epicentres, the increase of seismicity in Liaoning, the tilt at Chin Hsien and the Liaoyang swarm—may all be explained by a deformation front that later triggered the Haicheng earthquake.

I have, therefore, proposed a plausible hypothesis for connecting the phenomena used to make the prediction and the occurrence of the earthquake, the success of the prediction being the strongest argument for this plausibility. In doing so I have proposed the existence of a previously unknown phenomenon, the underlying mech-

anism of which is not understood. Deformation fronts have twice been directly observed to propagate between distant strain and tiltmeters in Japan^{7,8}, however. Migrations of large earthquakes, commonly at velocities between 80 and 270 km yr⁻¹ (ref. 6), may be caused by propagating deformation fronts, which may result from mantle stress waves^{9,10}. If this phenomenon is common, it may prove a powerful tool for earthquake prediction.

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Control of grip and stick in cell adhesion through lateral relationships of membrane glycoproteins

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Although cell adhesion to substrate must ultimately depend on 'sticking' by physical forces at the outer surface, we suggest that control is exercised through the 'grip' of the cytoskeleton. Our experiments indicate that grip can be relaxed by agents which release surface glycoproteins from some form of side-to-side organisation, and can be consolidated by suitable cross-linking to augment this organisation.

ONE of many stimulating challenges to our understanding of the function of animal cells, is to establish the mechanisms by which perturbations at the cell surface are expressed as changes in the cytoskeleton and vice versa. We show here that the response of fibroblast cells to contact with an inert substratum provides a convenient and appropriate system for investigation. When suspended cells settle on a favourable surface, the round shape undergoes a remarkable transformation to a more complex angular form which is flattened against the surface^{1–4}. This involves the formation of a three-dimensional meshwork of fibrillar proteins that is organised in relation to areas of adhesion^{3–5}, so that external contact has triggered a change from an unstructured to a highly organised cytoskeleton. The converse process of detachment from a substrate, for example by trypsin or chelating agents, is equally revealing because the sequence of intermediate stages shows that the primary event is usually the disintegration of a structured cytoskeleton^{6,7}. The cell body rounds up and withdraws from the substrate, leaving sites of adhesion intact; the process does not start

with dissolution of 'the glue' because only later does the cell float away. Further evidence that cell adhesion can be influenced (strengthened or weakened) by perturbing inner as well as outer structures, is that detachment is retarded when the intracellular level of cyclic AMP is artificially raised⁸ to enhance the stability of cytoskeletal elements⁹, and is accelerated by drugs which destabilise these elements^{6,10}. Thus it is important to distinguish between contributions to cell adhesion from internal structures, which we refer to as 'grip', and those from external structures which we refer to as 'stick'. Included in the 'stick' component are all manifestations of physical attraction between the cell surface and the substrate.

Preliminary characterisation of stick and grip

To investigate further whether fibroblast cells are detached from serum-coated glass by mechanisms that involve primarily the lesion of stick, or primarily the lesion of grip, we first used the interference reflection microscope. This instrument^{12–14} can visualise the zones of closest contact between the cell and substrate. These zones at first form a sharply defined pattern of subunits, each about 1–5 μm in area (Fig. 1c, f). Detachment by warm EGTA leaves the zones well defined (Fig. 1f, g) as the cell retracts and the membrane shows pronounced motility and blebbing. We interpret this as a disruption of cytoskeletal stiffening, so that the cell can now peel from the substrate even though the configuration is unaffected at each area of actual contact. From the theory of physical adhesion¹⁵ we know that, other things being equal, a deformable cell would be detached more readily than a stiffened cell because a

deformable body can detach by a peeling mode which is not open to a stiff one. Possible mechanisms that could cause peeling are the cell's own tension on individual sites (shown for example by the ability to deform flexible substrates¹⁶ and by the sharp recoil when adhesions are broken

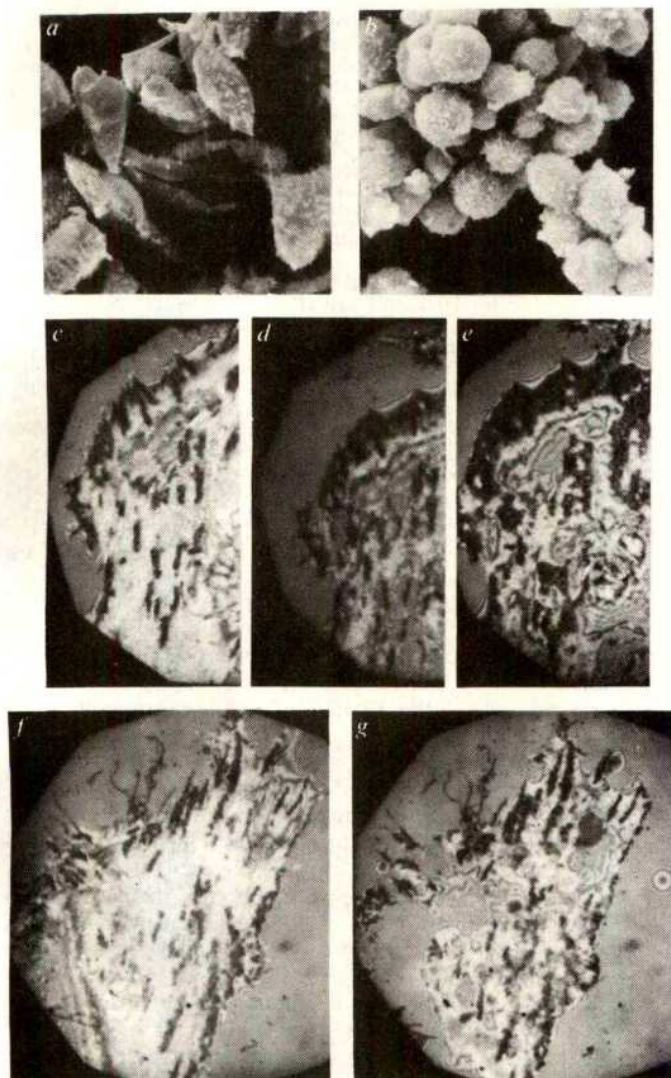


Fig. 1 Photomicrographs of rat dermal fibroblasts (16C line, Colworth strain) as they are influenced by various conditions of detachment from a glass substrate. *a*, Scanning electron micrographs ($\times 952$) of cells detached from a glass medicine bottle after decanting the medium, washing twice with ice-cold Hank's balanced salts solution (HBSS, Ca^{2+} and Mg^{2+} free), chilling in this solution for 15 min, then shaking off with ice-cold EDTA (0.02% in the same salt solution). Cells were immediately fixed by mixing with an equal volume of 3% glutaraldehyde in Dulbecco's phosphate-buffered saline (PBS). After 30 min at 0°C , the cells were centrifuged, washed twice with PBS, post-fixed with osmium tetroxide and prepared for microscopy by critical point drying. *b*, Cells as they appear after typical dissociation with trypsin (0.05%)—EDTA (0.01%) in PBS, or with EGTA (0.05%) in Tris-buffered Ca^{2+} and Mg^{2+} free saline, at room temperature. Washing, fixing and processing as above. *c, d, e*, Stages in detachment by trypsin ($100\ \mu\text{g ml}^{-1}$ in HBSS at 23°C) from a glass coverslip, as seen with interference reflection optics. Parts of the cell that are in close apposition to the glass surface (that is, the adhesion sites) appear as dense black zones. *c*, Before exposure to trypsin; *d*, after exposure for 7 min; *e*, after exposure for 15 min. Note that attachment sites are at first sharply defined and later spread and become continuous. For space reasons, only a part of the underside of one cell is shown. *f, g*, Stages in detachment by ethylene glycol bis (β -aminoethyl ether)-N, N-tetraacetic acid (EGTA, 0.05% in Ca^{2+} and Mg^{2+} free HBSS at 37°C). *f*, Before exposure to EGTA; *g*, after exposure for 35 min. Note that the size, shape, and mutual relationship of many attachment sites after little while an area of the cell on the left of the photograph peels away from the substrate.

by micromanipulation⁷), pinching off the adhesive plaques in an exocytotic-like process (we have seen such plaques left behind by cells, remaining attached and enclosed in membrane vesicles) and/or through any turbulence in the medium. Turbulence should also be more effective in detaching deformable cells because they generally present a higher profile to the shearing liquid.

The mechanism of detachment by trypsin is a little more complicated. As the cell slowly rounds under the action of trypsin, it leaves retraction fibrils to the attachment zone^{6,7}. We have found by interference reflection microscopy (Fig. 1*c, d, e*) that the zones themselves become less well defined and eventually merge into a continuous grey background. Although this might seem to suggest that trypsin has dissolved 'the glue' at each area of contact, such an explanation would be insufficient for the results of further experiments. Using procedures described in the legend to Fig. 2, we found that less than 10% of 16C cells were detached from a monolayer on glass by incubation with trypsin ($10\ \mu\text{g ml}^{-1}$) on ice for 40 min. If the trypsin was replaced at 25 min by soybean trypsin inhibitor ($200\ \mu\text{g ml}^{-1}$), however, and after a further 10 min in ice, allowed to warm to 37°C , almost all the cells detached immediately. Cells did not detach when cooled in the absence of trypsin and then warmed. Therefore this mild trypsinisation can cause the lesion that normally leads to detachment but this lesion is only expressed as a result of a cellular response made in the absence of trypsin and which requires metabolic activity and/or membrane fluidity. We suggest that trypsin impairs the cell's ability to hold surface macromolecules in an appropriate configuration at each area of contact—hence these configurations disperse and the organised adhesion is replaced by a more amorphous contact over a larger area.

Our provisional conclusion is therefore that adhesion is controlled by cellular activity (that is, by grip) in at least two ways (1) by the overall stiffness and shape which determines how easily the cell is peeled away and (2) by holding macromolecules in the appropriate organised configuration at areas of contact with substrate. We refer to these controls as 'bracing grip' and 'focal grip' respectively. EGTA seems to cause detachment by an action on the former, whereas trypsin has an action on both.

In passing, we point out (as others have^{9,11}) that conditions can be devised for detachment by lesion of 'stick' as well as 'grip'. Cells detached with cold EDTA or cold trypsin in more severe conditions than described above, can have a flat and angular form which is different from the round shape of those detached under conditions more typical of subculturing procedures (Fig. 1*a, b*). This shows that 'stick' between cell and substrate can sometimes be dissolved or broken by shear before the full alterations in the cytoskeleton which would lead to cell rounding.

Surface glycoproteins in the expression of grip

To investigate further the mechanisms of lesion of fibroblast grip to the substrate, we used plant lectins. These di- or multivalent carbohydrate-binding proteins are known to cross-link carbohydrate components of the outer cell surface, including those glycoproteins which pierce the membrane and are associated with the cytoskeleton. Much evidence^{5,17,18-20} shows that the movement of lectin receptors can be accompanied and sometimes even directed by changes in the cytoskeleton. Our results lead to a conclusion that is opposite to this but complementary—that constraints on lectin receptors can consolidate the cytoskeleton in a form that preserves grip to the substrate.

Several authors^{17,22-25} have previously stated or implied that lectins can inhibit the detachment of cells by proteases and chelating agents. Lectins which apparently have this property are concanavalin A (con A)^{22,23,25}, phytohaemagglutinin^{17,22}, and the lectin of higher molecular weight

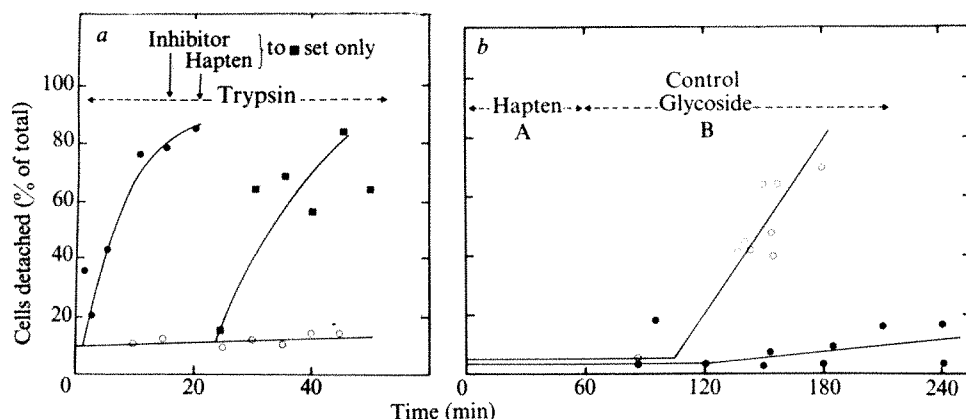


Fig. 2 Rate of detachment of fibroblasts from a glass substrate by trypsin, and its modification by con A. Established monolayers in glass Leighton tubes (without coverslips) were gently treated with the sequence of solutions indicated below. Each point of the graph represents the proportion of cells which could be detached (Coulter assay) by gently rolling the medium several times over the cells in a particular tube at a particular time, essentially as described by Johnson and Pastan⁸. *a*, Results for three sets of tubes of NIL 8 cells. Each tube of the first set (○), was gently washed with HBSS, incubated at 37 °C for 20 min with the same solution, then at 37 °C with crystalline trypsin (25 $\mu\text{g ml}^{-1}$) in HBSS. Each tube of the

second set (●), was gently washed with HBSS, incubated at 37 °C for 20 min with con A (100 $\mu\text{g ml}^{-1}$) in HBSS, then with trypsin as for the first set. Each of the third (■), was treated in the same way as the tubes of second set except that, after 15 min in trypsin, the cells were washed and incubated first with trypsin inhibitor (from soy bean, 100 $\mu\text{g ml}^{-1}$ in HBSS; 5 min at 37 °C) then with methyl α -D-mannoside (0.05 M in HBSS at 37 °C). All these operations and the results are shown schematically in Fig. 3. *b*, 16C cells were washed with HBSS, incubated at 37 °C with con A (100 $\mu\text{g ml}^{-1}$ in HBSS at 37 °C for 20 min), washed with HBSS and incubated with trypsin (25 $\mu\text{g ml}^{-1}$ in HBSS at 37 °C for 25 min), washed and flooded with trypsin inhibitor (from soy beans, 100 $\mu\text{g ml}^{-1}$ in HBSS) and then placed on ice for 10 min. The tubes were divided into two sets in this stage. One of the first set (○) were washed and incubated with ice-cold methyl α -D-mannopyranoside (0.05 M in HBSS). Tubes of the second (●) were washed and incubated with methyl β -D-galactoside (0.05 M) in HBSS. After 60 min, all cells (both sets) were carefully washed and flooded with ice-cold galactoside solution then allowed to warm to 37 °C. Note that the cold incubation with mannoside is necessary for subsequent detachment of cells in the warm with galactoside. In other control experiments, briefer treatment with mannoside (up to 30 min in the cold) was found to be insufficient for rapid detachment of a high proportion of cells when subsequently warmed with galactoside. The significant operations and results are shown schematically in Fig. 3.

from *Ricinus communis*²⁴. This has usually been explained in trivial terms, such as blocking of access to the detaching agent or the formation of lectin bridges between cell and substratum to replace the natural adhesions. We now describe experiments which show that these explanations are unlikely and then explore the implications of our own hypothesis, which has not previously been considered, namely, that adhesion is preserved by influencing the cytoskeleton through its relation with surface receptors that can be cross-linked by lectin.

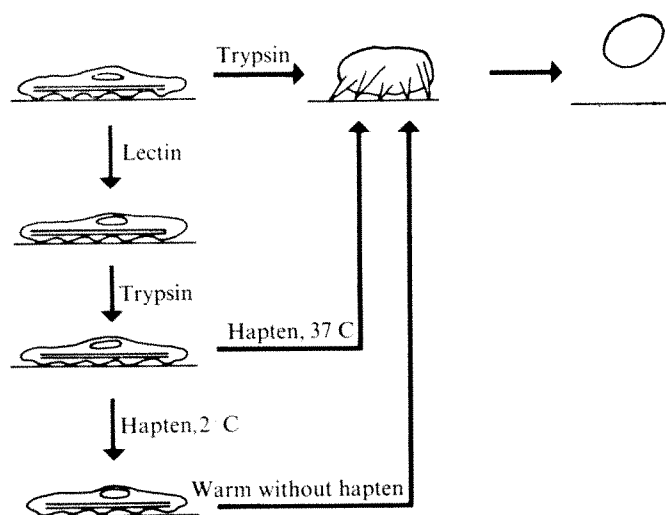


Fig. 3 Schematic representation of key experiments with lectins and cell detachment. The top line shows that cells normally round up with warm trypsin, leaving retraction fibrils which are then withdrawn as the cell separates from the substrate. The vertical sequence shows that pretreatment with con A prevents subsequent rounding and detachment under the influence of trypsin; after removal of the trypsin, however, displacement of con A at 37 °C (see first loop) causes rounding and detachment proving that the lectin block was in the expression of the tryptic lesion rather than at the level of the action of trypsin. The last step in the vertical sequence shows that the displacement of lectin from trypsinised, protected cells is not in itself sufficient to cause detachment—subsequent warming is necessary to re-start metabolic activity and loosen the grip.

Exposure of cells in monolayer to trypsin caused rapid rounding and detachment but when they had been pre-incubated with con A, very few were detached by this treatment. Even vigorous shaking and further amounts of enzyme produced little further effect. Measurements with ¹²⁵I-labelled con A showed that 16C cells bound 7.2×10^7 molecules of lectin per cell, most of which (5.7×10^7 molecules per cell) was inhibited in the presence of 0.05 M α -D-mannopyranoside. Trypsinisation (25 $\mu\text{g ml}^{-1}$ for 20 min at 37 °C) removed 32% of the specifically bound lectin, presumably as a combined result of cleavage of the lectin itself and of a proportion of the receptors for it. The con A-protected trypsinised cells could be detached readily by brief incubation with methyl α -D-mannoside (Fig. 2a). Since this effect was specific in that it was not observed with methyl β -D-galactoside, it is attributable to the displacement of the remaining bound con A and hence of its protective influence. These observations (shown schematically in Fig. 3) prove that target molecules have been split by trypsin in the usual way but that detachment was prevented by the bound lectin; the explanation of lectin protection in terms of mere blockage of the action of trypsin, is therefore untenable. Similar results but with interesting differences in detail (unpublished work in this laboratory), were obtained with EGTA detachment. Evidently both agents can act on lectin-coated cells to cause whatever lesion would normally result in cell detachment but the usual expression of each lesion is prevented until con A is displaced. We have observed that some (but not all) other lectins can act analogously to con A. The effect is observed with all the normal and transformed cell lines that we have studied.

After the action of trypsin, cell attachment is not retained merely through lectin bridges to the substrate (Fig. 3). If this were so, removal of these bridges would leave cells free to be washed away—whereas we find that they are still firmly attached after prolonged incubation with methyl α -D-mannoside in the cold (Fig. 2b). Measurements with ¹²⁵I-con A confirmed that the cold incubation was effective in displacing 62% of the bound trypsin-resistant lectin, which is evidently sufficient to allow the cells to detach when metabolic activity is allowed to re-start by subsequent warming to 37 °C in the absence of hapten (Fig. 2b). Cells did not detach extensively after shorter incubations with cold mannoside followed by warming after its removal (for

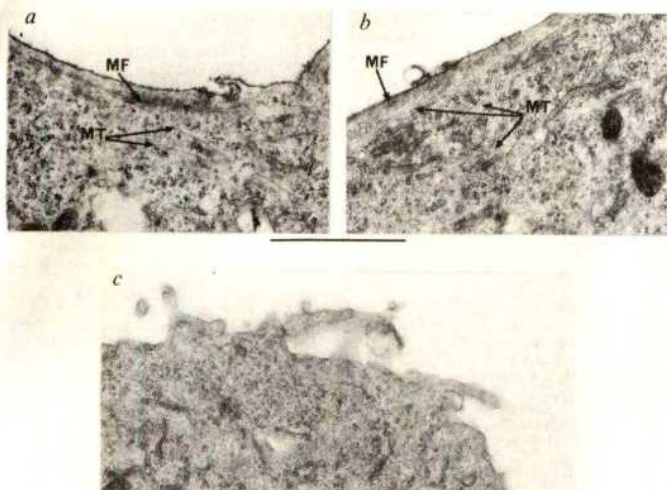


Fig. 4 Typical electron micrographs of BHK cells seeded singly and grown on melanex coverslips for 48 h. After appropriate treatment (see below), cells were fixed for 30 min at 37 °C in 3% glutaraldehyde with 4% tannic acid in 0.1 M cacodylate buffer pH 7.3, then washed briefly in 0.1 M cacodylate, and postfixed with 1% osmium tetroxide in 0.1 M cacodylate pH 7.3 for 30 min at room temperature then 1% aqueous uranyl acetate for 30 min. Dehydration was through a graded series of ethanol concentrations. The sample was embedded in Araldite and sections were cut parallel to the substrate and stained with lead citrate. *a*, Control BHK cell. *b*, BHK cell after incubation at 37 °C with con A (100 $\mu\text{g ml}^{-1}$ in HBSS) for 20 min followed by trypsin (25 $\mu\text{g ml}^{-1}$ in HBSS) for 30 min. *c*, BHK cell after incubation at 37 °C with trypsin (25 $\mu\text{g ml}^{-1}$ in HBSS) for 10 min. MT, microtubules; MF, microfilament bundles. Bar 1 μm .

example, after 30 min which removed only 30% of bound trypsin-resistant lectin).

Having discounted two explanations, we are left with the possibility that lectin constrains the lateral arrangement of glycoproteins in a way that preserves the integrity of cytoskeletal components responsible for grip. The rapid reversal of protective action when lectin is displaced in our

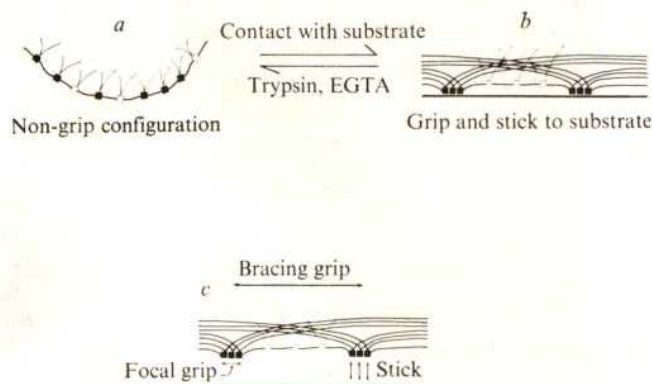


Fig. 5 Highly schematic model for lateral organisation of cell surface glycoproteins for grip and stick in cell adhesion to substrate—note the reservations in the text on molecular realism. *a*, The suspended cell is not organised intra- or extracellularly for adhesion. *b*, *c*, In the attached cell, surface molecules (■) have been moved and organised at the sites of adhesion in a way that sets up the development of the cytoskeleton; they are held in this configuration by focal grip. The full development of cell morphology requires further surface molecules (□) to be organised at sites outside the adhesions, in the process by which cell membrane is drawn down between adhesions to flatten the cell and stiffen it against peeling, that is, to maintain bracing grip. Cell rounding and detachment can be initiated by lesions in the natural side-to-side associations of either set of surface glycoproteins (■ or □); expression of either lesion is prevented by lectins which, by cross-linking the glycoproteins, suitably constrain their positions.

experiments by glycoside hapten confirms that effective binding is indeed on the outer membrane surface, rather than some result that follows endocytosis.

The cytoskeleton in the expression of grip

To characterise the response of the cytoskeleton to detachment agents and lectins, we followed changes in BHK cells that had been seeded singly and allowed to spread for 48 h. Thin sections cut parallel to the substrate showed that areas of spread membrane contained long microtubules and bundles of microfilaments close to the underside of the cell (Fig. 4), and were bounded by a stretched, linear cell margin. By contrast, light microscopy showed trypsinised cells to be rounded and blebby, and thin sections confirmed this loss of surface tone. The convolution and infolding of the cell's margin is accompanied by progressive fragmentation and the eventual disappearance of microfilament bundles and microtubules prior to cell detachment (Fig. 4). When cells had been pretreated with con A, however, these trypsin-induced changes were arrested at an early stage (Fig. 4). Although cell shape may be altered, the margins remained generally smooth, long microtubules remained in most cells, and microfilament bundles could be observed in many.

From these experiments we can therefore confirm that pretreatment with con A prevents the action of trypsin from causing not only cell detachment but also the dissolution of cytoskeleton. There is some indication that con A might be more efficient in arresting the dispersal of microtubules than of microfilament bundles but the evidence for this is not clear cut. For the time being at least we prefer to regard these components as linked in a single system which is perturbed together by the agents we have used.

Model for glycoprotein relationships in stick and grip

We conclude that lectin cross-linking of carbohydrate chains on the outer surface can constrain the cell in a way that keeps it flattened and attached to the substrate, despite the action of agents that normally cause it to withdraw and detach. Whether the target sites for these agents are inside or outside the cell, the lesions they induce are not expressed until after the receptors have been released to rearrange in the plane of the membrane. At least two types of lesion can be involved in cell detachment: dispersal of the adhesive areas, for example by trypsin, and loosening of the cell body without changing the form and distribution of these areas, for example by EGTA. In our terms these represent lesions in focal grip and bracing grip respectively. The expression of both can be blocked by appropriate lectins.

We now propose a model to account for these results. In the suspended cell, surface receptors are not organised (Fig. 5, non-grip configuration). The development of the stiff, flat and attached form of the cell, however, requires lateral organisation of surface glycoproteins for the functions of focal grip and bracing grip (Fig. 5). Note that this representation is highly schematic and does not attempt to show the relative dimensions of molecular components of the surface and cytoskeleton (compare with ref. 26) or indeed to distinguish between different components of the cytoskeleton such as microfilament bundles and microtubules.

The relevant glycoproteins are exposed on the outer surface and pass through the lipid membrane to integrate with the cytoskeleton in such a way that ordering of the cytoskeleton necessarily involves an ordering of the lateral arrangement of these glycoproteins and likewise for dis-ordering. In the morphologically developed cell, specific side-to-side organisation of these glycoproteins is presumably maintained by natural bridging molecules, inside and/or

outside the membrane—and these are targets for agents which cause detachment. Such bridging molecules have yet to be characterised but submembranous, fibrillar proteins and external, endogenous, trypsin-sensitive, agglutinins^{28,29} could clearly be involved.

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letters to nature

Cosmological tests of general relativity

GENERAL relativity theory could be tested on a cosmological scale by measuring the Hubble constant and the deceleration parameter, if, in addition, everything could be known about the matter filling the universe. If, on the other hand, nothing could be presupposed about the matter content of the universe, general relativity could not be tested by measuring any number of time derivatives of the scale factor. But upon making the assumption of a universe filled with a non-interacting mixture of non-relativistic matter and radiation we can in principle test general relativity by measuring the first five derivatives of the scale factor. In the following, some general relations are presented using this assumption.

A reasonable model for the universe in a not too early stage of its development is the Robertson-Walker metric

$$ds^2 = -dt^2 + R^2(t) [(dr^2)/(1-kr^2) + r^2 d\Omega^2] \quad (1)$$

describing a homogeneous isotropic universe.

From general relativity theory it follows that the scale factor $R(t)$ obeys the Friedmann equation

$$\dot{R}^2 + k = \frac{8\pi G}{3} R \rho + \frac{\lambda}{3} R^2 \quad (2)$$

and conservation of energy and momentum is expressed by

$$\frac{d}{dR} (\rho R^3) = -3pR^2 \quad (3)$$

Here and throughout this paper we put $c = 1$; λ stands for the cosmological constant, while ρ and p denote the density and pressure of the matter content of the universe.

To test these equations we need observations. By measuring the luminosity distance or the angular diameter distance of remote objects as a function of redshift z , we can in principle determine the Hubble constant $H = \dot{R}/R$, the deceleration parameter $q = -\ddot{R}R/\dot{R}^2$, and an indefinite number of similar dimensionless parameters constructed from higher time derivatives¹. We shall use here, following Harrison²

$$Q = \frac{\ddot{R}R^2}{\dot{R}^3} \quad X = \frac{R^{(4)}R^3}{\dot{R}^4} \quad Y = \frac{R^{(5)}R^4}{\dot{R}^5} \quad (4)$$

(all evaluated at the present time, with signs which make them positive for vanishing λ , as will be shown). Without using Friedmann's equation (2), however, knowledge of any number of those parameters will not suffice to obtain the value of the present curvature $K = k/R^2$ (ref. 3). But even equations (2) and (3) are not sufficient without additional information about the equation of state, as can be seen as follows: by differentiating equation (2) N times we obtain $(N+1)$ equations for $(N+2)$ unknowns: ρ and its first N derivatives and λ .

A plausible form for the equation of state is obtained by assuming that at present the universe contains a non-interacting mixture of two forms of matter, non-relativistic matter with density ρ_m and ultra-relativistic matter with density ρ_r . Then

$$\left. \begin{aligned} \rho &= \rho_m + \rho_r \\ p &= \frac{1}{3} \rho_r \end{aligned} \right\} \quad (5)$$

For the non-relativistic matter, density, particle number conservation gives

$$\rho_m = A/R^3 \quad (6)$$

and thus with (3)

$$\rho_r = B/R^4 \quad (7)$$

where A and B are constant.

Now, carrying out three successive time differentiations of equation (2) leads with (2) to four equations for the newly introduced time derivative parameters. Solving these for the quantities K , λ , ρ_m and ρ_r , gives

$$K = \frac{1}{4} (-X + 4Q + Qq + 2q - 2) H^2 \quad (8)$$

$$\lambda = \frac{1}{4} (-X + 6Q + Qq - 6q) H^2 \quad (9)$$

$$\rho_m = \frac{1}{4\pi G} (-X + 3Q + Qq + 3q) H^2 \quad (10)$$

$$\rho_r = \frac{3}{32\pi G} (X - 2Q - Qq - 2q) H^2 \quad (11)$$

Differentiating (2) a fourth time finally leads to a relation

between the four parameters q , Q , X and Y which should hold if general relativity is valid

$$Y = 7X + 3Xq - 6Q + Q^2 - 7Qq - 3Qq^2 - 6q \quad (12)$$

This condition provides (in principle) a full test of the theory.

If less than five parameters measuring the time derivatives of the scale factor are known—as certainly will be the case in practice—still conditional relations can be derived between the various parameters. From equations (10) and (11) it follows that, since $\rho_m > 0$ and $\rho_r > 0$

$$Qq + 2(Q+q) < X < Qq + 3(Q+q) \quad (13)$$

a condition valid if only q , Q and X are known.

If X is not available either we introduce a new parameter α , still to be able to express the four quantities K , λ , ρ_m and ρ_r explicitly. For α we take

$$\alpha = \rho_r / (\rho_m + \rho_r) \quad 0 \leq \alpha \leq 1 \quad (14)$$

and we then obtain

$$K = [-1 + (3 + \alpha)/(3 + 5\alpha) Q - (4\alpha)/(3 + 5\alpha) q] H^2 \quad (15)$$

$$\rho = 1/8\pi G 6/(3 + 5\alpha)(Q + q) H^2 \quad (16)$$

$$\lambda = 3[(1 + \alpha)/(3 + 5\alpha) Q - (2 + 4\alpha)/(3 + 5\alpha) q] H^2 \quad (17)$$

Equation (16) gives us, as $\rho > 0$, the condition

$$Q + q > 0 \quad (18)$$

If we only know H and q —a situation which is at present striven for—it is regrettably no longer possible to write down even conditional equations, unless observational evidence of a different nature, for example, regarding the age of particular objects in the universe, is taken into account.

Figure 1 illustrates a classification, based on equations (15) and (16), of the structural and evolutionary properties of the universe, as depending on the values of q and Q , but with arbitrary λ . We can distinguish between domains of positive and negative ρ (the latter physically unrealistic), of $k = \pm 1$ (separated by a line $k = 0$), and the presence or absence of an initial and a final singularity (only with a final singularity the universe, at present expanding, has a finite lifetime). Depending on the value of α , the relative amount of matter which is present in the form of radiation, the borderlines between regions may change. Their equations are given in the two extreme cases $\alpha = 0$ and $\alpha = 1$ as

$$Q = q^2 + q + 1; \quad \alpha = 1 \quad (19)$$

$$Q = \frac{1}{3} \left[2q^2 + q + 2 + 2 \left(q^4 + \frac{8}{3}q^3 + 2q^2 - \frac{1}{3} \right)^{1/2} \right]; \quad \alpha = 0$$

Where the status of the universe depends on α , this is indicated by a double label as explained in the caption. Comparable diagrams have been published earlier by Stabell and Refsdal⁴. (As a referee commented, Fig. 3 of ref. 4, which is a σ - q diagram, is topologically very similar to Fig. 1 of this paper, as $\sigma = 4\pi G\rho/3H^2 \sim Q + q$.)

We can further simplify equations (8)–(11) by introducing two transformed time-derivative parameters

$$\left. \begin{aligned} \tilde{Q} &= Q + q \\ \tilde{X} &= X - Qq \end{aligned} \right\} \quad (20)$$

Then the right-hand side of equations (8)–(11) can be seen to

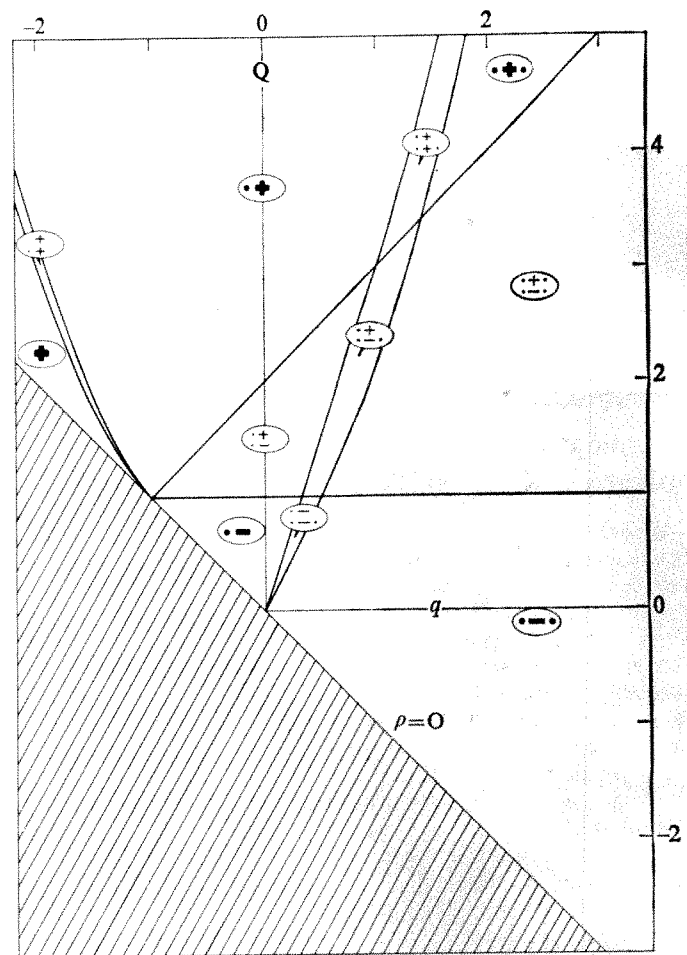


Fig. 1 Classification of structural and evolutionary properties of the universe in terms of Q and q with arbitrary λ . The shaded area is unphysical since $\rho < 0$ there. The symbols in the other regions have the following meaning: if one plus or minus sign is given, the value of the topological parameter k is $+1$ or -1 , respectively. A dot to the left of the sign denotes the presence of an initial singularity, and a dot to the right a final singularity. If a region has only one label, the conclusions follow irrespective of the value of α . If it bears two labels, the upper label denotes the situation for pure non-relativistic matter ($\alpha = 0$) and the lower one applies to the case of pure radiation ($\alpha = 1$). If $0 < \alpha < 1$, as is the case in our universe, the border lines lie always somewhere in between those given by the two extremes $\alpha = 0$ and $\alpha = 1$.

become linear expressions in the new parameters, where equation (12) transforms into

$$Y = 7\tilde{X} + 3\tilde{X}q - 6\tilde{Q} + (\tilde{Q} - q)^2 \quad (12')$$

The conditional relations (13) and (18) become very simple

$$2\tilde{Q} < \tilde{X} < 3\tilde{Q} \quad (13')$$

$$\tilde{Q} > 0 \quad (18')$$

We conclude that surprisingly simple relations can be derived, on the basis of rather general assumptions regarding the structure and matter content of the universe, between the 'physical' parameters of the universe ρ_m , ρ_r and λ , together with K , and the 'geometrical' parameters involving the first four time derivatives of the scale factor. Between the first five time derivatives a strict relation should hold if general relativity applies. This relation forms, in principle, a definite

test for the theory. If less than five parameters are known, but more than two, conditional relations can be derived.

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Tugboat model for OB binaries, X-ray stars and pulsars

AN examination of the kinematical properties of binary OB stars, binary X-ray sources and pulsars suggests an evolutionary sequence linking an apparent low-velocity class of pulsars to the binary nature of their extreme Population I progenitors.

In a compilation of OB supergiants by Hutchings¹, 10 of the 65 stars listed are known binaries, and several more have variable velocities indicating a possible binary nature. Table 1 summarises the kinematical properties of these systems, grouping them according to the nature of their companions—four which have normal stars as secondaries (that is unevolved systems), three which have invisible, low-mass companions and three which are known X-ray sources presumably containing either a neutron star or a black hole. Comparison of columns 3 to 5 of Table 1 reveals distinct kinematical differences among these three groups. The unevolved systems have low space velocities and z -heights typical of extreme Population I objects. The group of stars with unseen, low-mass companions have similar z -heights, but much higher velocities. Their low- z values indicate that these velocities must have been acquired recently, for such speeds would indicate origins far (> 500 pc) from the plane if they were to be maintained on evolutionary timescales ($\sim 10^7$ yr). The velocities for the X-ray sources are not well determined, but their distances from the plane are larger than that for typical extreme Population I objects.

A careful comparison of the z -distribution of pulsars with their characteristic ages given by $\tau = P/2\dot{P}$ yields several clues about the kinematics and evolution of the pulsar population. The pulsar scale height $z_{r.m.s.} \approx 250$ pc (ref. 8) indicates that these objects must in general have velocities much larger than those of the massive OB stars ($z_{r.m.s.} \approx 80$ pc) from which they

are thought to originate. Recent measurements of pulsar proper motions^{9–12} have confirmed this conclusion, finding transverse velocities ranging from 50 km s^{-1} to well over 500 km s^{-1} . For $\tau < \sim 5 \times 10^6$ yr, the observed z -distribution, properly corrected for selection effects¹³, shows a rise of scale height with age that is consistent with the observed pulsar velocities. There is, however, a significant fraction of pulsars ($\sim 25\%$) with much larger characteristic ages, whose z -distribution implies absurdly low velocities (often with $v_z \ll 1 \text{ km s}^{-1}$). Proper motion measurements for several of these sources have yielded much higher velocities, showing that the characteristic age is not a true indicator of chronological age after a few Myr, thus confirming such suggestions made earlier from other evidence^{14,15}.

The discrepancy between dynamical and characteristic ages may be related to the decrease in the quantity $P\dot{P}$ ($\propto B^2 R^6 / I$) with time, perhaps as a result of magnetic field decay^{14,15}, alignment of the pulsar's magnetic and spin axes¹⁶, or some other process. One can model such a decay as an exponential decrease in $P\dot{P}$ with a characteristic timescale τ_D given by the time at which the characteristic and dynamical ages diverge ($\tau_D \sim 5 \times 10^6$ yr), and thus derive a new age estimate for each source, as well as the quantity $P_0\dot{P}_0$ proportional to the sources' initial magnetic field strength and moment of inertia. Comparison of $P_0\dot{P}_0$ with measured pulsar velocities shows that the three sources (Class A) with the lowest initial fields have $\langle v_t \rangle_A \lesssim 60 \text{ km s}^{-1}$ while the remaining nine sources (Class B) all have $v_t > 100 \text{ km s}^{-1}$ with $\langle v_t \rangle_B \sim 265 \text{ km s}^{-1}$. Furthermore, from the much larger sample of sources with measured values of P , we find that the 18 sources with small $P_0\dot{P}_0$ have a mean $|z|$ of ~ 75 pc, while the 59 sources with larger $P_0\dot{P}_0$ have $\langle |z| \rangle_B \sim 250$ pc (ref. 13).

We have proposed an explanation of these two dynamical classes of pulsars in terms of the different environments in which they originate¹³. The high-velocity, widely distributed sources are supposed to originate primarily from single stars. They were presumably accelerated by means of the asymmetric radiation force¹⁷, which provides a natural explanation for their wide range of velocities through small variations in the asymmetry coefficient. The second class of pulsars is presumed to arise in tightly bound, massive binaries where the asymmetric force is insufficient to unbind the system (the supernova explosion that produces the neutron star will not usually unbind the system either¹⁸). The force thus acts on the system as a whole, accelerating it to a velocity that is reduced from that which the free neutron star would have attained by the ratio of the stellar masses (typically $\lesssim 0.1$). The neutron star thus acts as a tugboat: it pulls an object more massive than itself, and thus its own velocity is diminished. When these neutron stars are eventually

Table 1 Binary kinematic data

	Star	D (kpc)	z (pc)	V_t^* (km s^{-1})	$V_r^\dagger$ (km s^{-1})	P (d)	Companion	Refs‡
I	HD 36486	0.3	90	5	+16	5.7	B2 V	2
	HD 57060	0.9	80	30	—11	4.4	Equal mass companion	2
	HD 163181	0.8	55	20	—40	12.0	Possibly O9	3
	HD 166937	0.15	5	4	—6	180.0	Eclipsing	2, 4
II	HD 108	1.0	30	110	—70	4.6	Low mass, invisible	2, 5
	HD 148937	0.8	20	(80)	—	—	Low mass, invisible	2, 6
	HD 152667	1.3	30	75	—35	7.8	Low mass, invisible	2
III	HD 77581 (3U 0900-40)	1.3	140	(100)	+20	8.95	Pulsating X-ray source	6, 7
	HD 153919 (3U 1700-37)	1.2	65	(60)	—65	3.4	Pulsating X-ray source	6, 7
	HD 226868 (Cyg X-1)	2.0	140	(280)	—2	5.6	High mass X-ray source	6, 7

*Transverse velocities in parentheses are only $1.5\text{--}2.0\sigma$ PM results from ref. 6.

†Radial velocities for OB supergiants may be strongly affected by photospheric expansion.

‡All information not in the cited references is from ref. 1.

released at the time of the second supernova, they will already be several Myr old. While passing through the X-ray binary stage they will have undergone a spin-down due to magnetospheric braking in the stellar wind of the companion and a subsequent spin-up to a period of $\lesssim 1$ s due to accretion torques (compare the evolutionary model for such massive systems of van den Heuvel¹⁹). Thus it is not surprising that their characteristic and dynamical ages disagree. They will be relatively low-velocity objects, with final space velocities composed of their former systemic and orbital velocities. The high orbital velocities observed for some of the neutron stars in X-ray binaries are apparently reduced by mass loss from the system before disruption. The system will apparently be disrupted when the second supernova occurs (the relative infrequency of binary pulsars attests to the high probability of disruption) and the second remnant neutron star will be accelerated by its asymmetric radiation force to a high velocity and join the Class B sources.

In this picture, the 10^3 -yr-old Crab pulsar at $z = 200$ pc and $v_r > 100$ km s⁻¹ is a result of the second supernova that disrupted the system which was carried to this height by the acceleration of the remnant from the earlier explosion. The binary pulsar system also apparently reached its present $z = 230$ pc in a similar manner, but the evolution of its companion has failed to disrupt the system. Many of the OB runaway stars which have typical kinematical ages of 1–5 Myr (ref. 20) may be binary systems that have been accelerated by the primary remnant, but have yet to reach the second supernova stage. The high velocity group 2 sources in Table 1 are a subset of these stars for which the existence of a low-mass companion has been established. They will turn on as X-ray sources a few Myr (ref. 19) later at higher z (group 3).

The fact that even the youngest pulsars and a large fraction²¹ ($\sim 30\%$) of the radio supernova remnants are found at $z > 100$ pc is consistent with a mechanism that drags some OB stars away from their birthplaces before they become supernovae.

The relative frequencies of OB binary systems, high latitude SNRs²¹, and Class A pulsars¹³ are all 20–30%; in addition, the total galactic population of Class A pulsars and systems able to evolve through the X-ray binary stage in $\sim 10^7$ yr are also in reasonable agreement. Future observations of more pulsar proper motions and searches for pulsars, X-ray sources and/or hidden, low-mass objects as companions to OB stars could better define this evolutionary picture.

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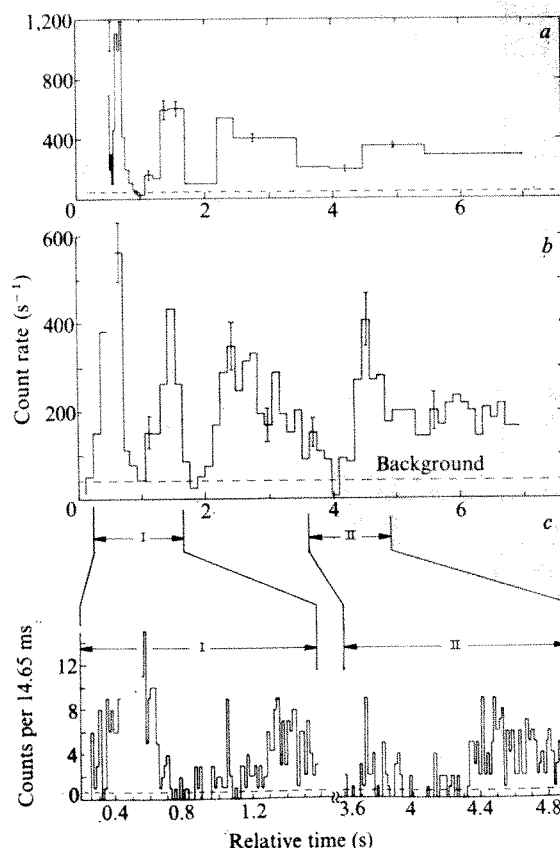
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Preliminary results from Solrad 11 γ -burst detectors

WE present here temporal and 0.2–2 MeV spectral data from two γ bursts observed on 12 June and 16 August 1976, by detectors on the Solrad 11A and 11B satellites. The 12 June burst showed evidence for structure on time scales down to ~ 10 ms throughout its lifetime, whereas the 16 August burst varied only with characteristic times longer than a few tenths of a second. A search for both slow and fast spectral variability gave negative results. Accurate absolute burst times are, however, not yet available, but since both bursts were also observed by at least one Vela satellite, positions are calculable and will be reported.

The Solrad 11A and 11B spacecraft were launched in March 1976, and are currently positioned on opposite sides of the Earth at heights of about 20 Earth radii. The γ -burst instrument on each spacecraft is omnidirectionally sensitive over the 0.2–2 MeV range and consists of a pair of $1\frac{1}{2}$ inch by $1\frac{1}{2}$ inch CsI scintillation detectors, the combined output of which drives circuitry designed for response only to rapid, transient events. A total average count rate during any 0.625 s interval of greater than about 7 sigma above background triggers the system, causing temporal and spectral information—including 0.6 s of pre-trigger data—to be stored in a memory for later telemetering to Earth. The temporal information is stored as data words which can be either of two types. For count rates below $1,092$ s⁻¹ the total number of counts accumulated per 14.65 ms is

Fig. 1 Count rate plotted against time for the 12 June burst. The time origin of the graphs is at $1903:40$ UT ± 2 s. *a*, Response of Vela 5A, which triggered at about the same time as Solrad 11A, but which does not store pre-trigger data. The full time resolution capability of this and the other Vela detectors is displayed here. *b*, Eight-channel (117.2 ms) time averages of Solrad 11A data for the same burst. *c*, Segments I and II of the Solrad data, but with 14.65 ms resolution.



recorded. At higher rates the time interval required to accumulate 16 counts is recorded with 0.3 ms resolution. Counts are simultaneously accumulated into four spectral channels covering the energy ranges of approximately 0.2–0.3, 0.3–0.4, 0.4–0.6, and 0.6–2.0 MeV. The contents of the spectral channels are read into the memory after each eight time accumulations, so the time resolution for spectral changes can range from 2.4 to 117.2 ms. Since there is no provision for in-flight energy calibration and the spacecraft mass complicates the detector response, the spectral data can be used to search for spectral variability, but not to obtain reliable spectral shapes.

Figure 1 shows count rate plotted against time for the burst of 12 June. Since the detector high voltage on Solrad 11B was turned off at the time of this burst, the only data available are from Solrad 11A and the Vela satellites. We have used two different time scales to show the overall burst structure and to give a higher time resolution view of selected portions of the burst. The 12 June burst was relatively intense, and was observed by γ -burst detectors on four Vela satellites. Figure 2 is a similar presentation of the 16 August burst. The response of 11A is low relative to 11B due to the probable malfunction of one of its two CsI detectors. This burst is small by Vela standards, having been just intense enough to trigger one Vela detector.

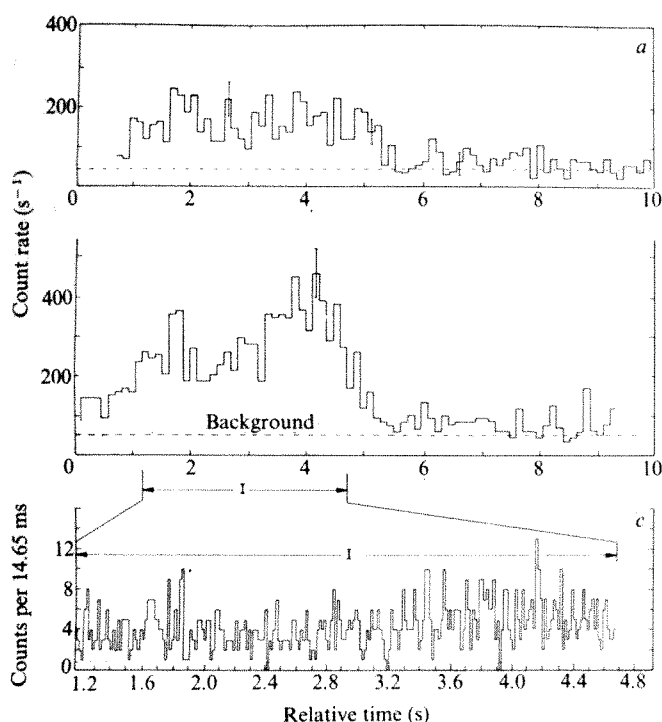


Fig. 2 Count rate plotted against time for the 16 August burst. The time origin is at 1615:31 UT $\pm$ 2 s. *a* and *b*, 117.2-ms averages of the responses of Solrad 11A and 11B, respectively. *c*, Section I of the Solrad 11B data replotted with 14.65-ms resolution.

The high time resolution graphs (Figs 1*c* and 2*c*) demonstrate an important difference in the signatures of the two bursts. The 12 June burst is very 'spiky', and shows clear evidence for structure on time scales as fast as ~ 10 ms. The fact that finer structure is not seen might simply be due to insufficient detector sensitivity, since the time resolution becomes better than 14.65 ms only for count rates higher than $1,092 \text{ s}^{-1}$. The 16 August burst, on the other hand, shows statistically significant structure only on time scales slower than a few tenths of a second. Since the two bursts were detected at about the same statistical level, the apparent difference between them is almost certainly real.

We have not explicitly shown rates in any of the separate spectral channels, but we have examined these data for evidence of spectral variability. Specifically, we carried out a χ^2 test of

the assumption that the hardness ratio—that is, the ratio of the flux in channels 3 and 4 to the flux in channels 1 and 2—remained constant throughout each burst. That is, we examined the quantity

$$\chi^2 = \sum_i \left(\frac{r_i - R}{\sigma_i} \right)^2$$

where r_i is the hardness ratio in a given spectral accumulation period, σ_i is an estimate of the standard deviation of r_i , and R is a free parameter that is the hardness ratio averaged over the burst. In several instances the probability distribution functions of the r_i differed significantly from the normal probability distribution, thus complicating the χ^2 analysis. (Note that a random variable that is the ratio of even normally distributed random variables is not approximately normally distributed unless the denominator in the ratio has a small fractional standard deviation.) Although we accounted for this effect in the worst cases—that is, r_i calculated from the smallest statistical samples—the χ^2 values obtained should be considered as only approximate indicators of goodness-of-fit.

To carry out the most sensitive possible search for both slow and fast variability, we carried out various time averages of the data before applying the χ^2 test. Basically, we found no compelling evidence for spectral variability, although for some of the time averages in the 0.25–1 s range χ^2 probabilities were below $\sim 10\%$. Since the spectral variability suspected on the basis of these probabilities was not correlated with the overall temporal structure of the bursts, however, we felt that the low probabilities were due partly to chance and partly to the statistical complications discussed above. The uncertainty in R for either burst was 5%, implying spectral constancy to within $\sim 10\%$ on several-second time scales.

In summary, we have seen evidence in one of two γ bursts for temporal structure on time scales down to ~ 10 ms. Higher detector sensitivity might have revealed faster time structure. We have also seen evidence that over the 0.2–2 MeV range the spectra of the two bursts were constant on several-second time scales to within $\sim 10\%$ during the observed—that is, most intense—portions of the bursts. This evidence favours theoretical burst models having the following features: The emission region can be at least as small as a white dwarf. The temporal smoothness of the 16 August burst suggests, however, that some burst sources might be much larger in at least one dimension, or that the γ rays sometimes undergo considerable dispersion—for example, by Compton scattering—between the emission region and the Earth. The source of the burst energy is not much affected by the burst itself, nor does it seem that the source is undergoing rapid physical change during the course of the burst. That is, we do not see evidence for physical processes such as rapid cooling or magnetic field changes that would be expected to cause spectral variations.

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Detection of ^4He in stratospheric particles gives evidence of extraterrestrial origin

WHIPPLE predicted the existence of micrometeorites, small interplanetary particles which enter the Earth's atmosphere without being melted by frictional heating¹. During the past 2 yr, large numbers of micrometre sized stratospheric particles have been collected which are believed to be extraterrestrial, because their elemental compositions closely match those of primitive meteorites^{2,3}. We report here the detection of large concentrations of ^4He in some of the particles. This not only suggests an exposure to solar wind, but also indicates that these particles are true micrometeorites in the sense that they were not strongly heated by entry into the atmosphere. Strong heating would have caused much of the helium to escape.

The particles were collected by an inertial impaction collector attached to a NASA U-2 aircraft flown at 20 km. The collector consisted of a 20-cm² oil-coated collection surface which was rammed through ambient air at the aircraft's cruise speed of 200 m s⁻¹. With the exception of aluminium oxide, a common stratospheric contaminant in this size range that is thought to come from solid fuel rocket propellant⁴, more than half the particles collected are believed to be of extraterrestrial origin. Nondestructive X-ray analyses have shown that most of these particles have abundances of Fe, Mg, Si, C, S, Ca, Al, Ni, Cr and Mn which closely match those of type C-1 carbonaceous chondrite meteorites^{2,3}. A total of 250 particles with diameters $\geq 4 \mu\text{m}$ have been collected by sampling about $3 \times 10^3 \text{ m}^3$ of stratospheric air.

Surfaces exposed to the interplanetary medium usually contain relatively large amounts of ^4He due to implantation of solar wind⁵. Ions from solar wind implant to depths of $\sim 500 \text{ \AA}$ in solid surfaces and form helium-rich layers on all exposed areas. Typical lunar soils have solar wind contents of the order of $0.1 \text{ cm}^3 \text{ (STP) g}^{-1}$. Lunar soil breccias and gas-rich meteorites also contain solar wind helium, but at generally lower levels due to diffusive losses and other processes. The flux of ^4He from the solar wind, as measured from the Apollo solar wind foil experiments⁶, varies from 4×10^6 to $18 \times 10^6 \text{ cm}^{-2} \text{ s}^{-1}$. From these measurements, it can be calculated that for $10\text{-}\mu\text{m}$ -sized grains exposed to solar wind at 1 AU, a saturation level

of helium would be reached on a time scale of 10–100 yr. The level of saturation depends on the grain temperature and mineralogy, but is expected to be in the range 0.01 to $1 \text{ cm}^3 \text{ (STP) g}^{-1}$.

To verify the extraterrestrial origin of the collected stratospheric particles, ten were analysed for helium using a high-sensitivity gas mass spectrometer. Seven of the ten provided measurable quantities of ^4He (Table 1). Although in several cases the helium concentration levels were quite high, the absolute amounts of helium measured were always small, of the order of 10^9 – 10^{10} atoms, which made it necessary to use the specialised mass spectrometer system and special techniques.

The stratospheric particles were prepared for helium analysis by mounting them individually in small aluminium foil sandwiches. Particles were transferred to the foils under a stereo microscope using a hand-held $8\text{-}\mu\text{m}$ diameter glass fibre. Silicone oil was used in the transfer and $\sim 10^{-10} \text{ g}$ of residual oil was retained on each particle. After particle transfer, each foil was folded to form a sandwich to enclose and protect the particle. The mass of aluminium ranged from 2×10^{-5} to $7 \times 10^{-5} \text{ g}$. In addition to the ten foil sandwiches which contained particles, fourteen sandwiches containing only the silicone oil drops were also prepared for use as control samples. For each set of mass spectrometer runs, the masses of the aluminium control foils closely matched the masses of the sample foils.

The mass spectrometer system we used⁶ involves no new concepts, but has refinements over other systems^{7–9}, giving simplicity of operation and an ability to detect low concentrations of helium in small specimens. Helium content was determined by melting and vaporising each aluminium foil packet in a resistance-heated tungsten coil under a vacuum. The released helium ($\sim 10^9$ atoms ^4He) was quickly passed over liquid nitrogen-cooled activated charcoal and non-vaporisable Al–Ti getters operated at room temperature⁶. Two minutes after vaporisation, a known fraction of the released helium gas was allowed directly into the mass spectrometer volume which had just been isolated from its vacuum pumps for 'static mode' operation. Absolute calibration of the instrument sensitivity was accomplished by adding a precisely known number of atoms of ^3He ($\sim 1 \times 10^{13}$ atoms) to the sample vacuum enclosure, and following the identical procedure to introduce the gas to the mass spectrometer. These low level ^3He spikes, which

Table 1 Helium measurements in stratospheric particles

Sample No.	Estimated mass (ng)	Measured amount of ^4He and 1σ uncertainty (in units of 10^9 atoms)			Net ^4He released by particle	Concentration of ^4He in particle ($\text{cm}^3 \text{ (STP) g}^{-1}$)
		Released by particle, plus system background	Released by control, plus system background	Range of system background		
SP-2	0.34	5.7 ± 0.2	3.4 ± 0.2	3.2–3.5	2.3 ± 0.3	0.25 ± 0.03
SP-3	0.43	3.8 ± 0.2	3.4 ± 0.2	3.2–3.5	0.4 ± 0.3	0.035 ± 0.026
SP-9	0.33	3.6 ± 0.1	2.8 ± 0.2	2.7–3.0	0.8 ± 0.2	0.09 ± 0.02
SP-10	2.0	4.4 ± 0.1	2.8 ± 0.2	2.7–3.0	1.6 ± 0.2	0.030 ± 0.004
SP-11	0.55	3.4 ± 0.2	3.5 ± 0.2	3.0–3.6	–0.1 ± 0.3	≤ 0.02
SP-12	1.0	3.2 ± 0.2	3.5 ± 0.2	3.0–3.6	–0.3 ± 0.3	≤ 0.01
SP-14	6.8	30.2 ± 0.2	2.9 ± 0.1	2.7–3.0	27.3 ± 0.2	0.149 ± 0.001
SP-15	24	3.3 ± 0.2	2.0 ± 0.2	1.8–2.2	1.3 ± 0.3	0.0020 ± 0.0003
SP-16	0.79	2.0 ± 0.2	2.0 ± 0.2	1.8–2.2	0.0 ± 0.3	≤ 0.01
SP-17	0.21	3.2 ± 0.1	1.9 ± 0.1	1.7–2.0	1.3 ± 0.2	0.23 ± 0.04

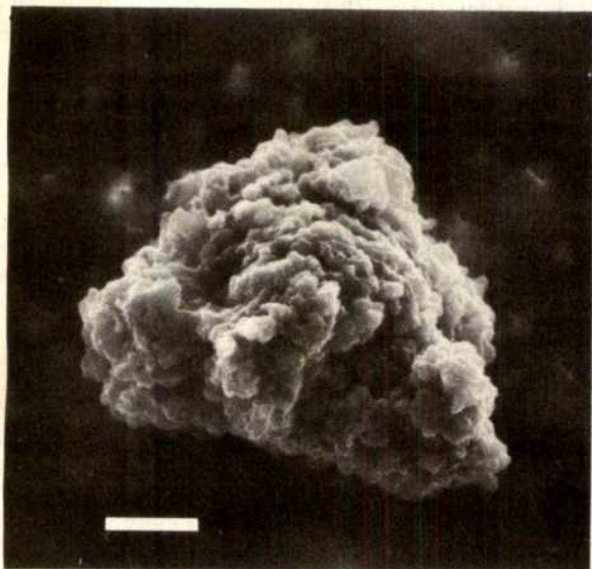


Fig. 1 Scanning electron micrograph of stratospheric micrometeorite SP-14. The elemental abundances of the particle match type 1 carbonaceous chondrite meteorite values within a factor of two, and as revealed at higher magnification, the particle is an aggregate of $\sim 1,000$ -Å grains. The scale bar represents 5 μm .

were obtained by partitioning through calibrated volumes, were preferred to ^4He because of possible 'memory' effects that the latter isotope might contribute. The usually preferred 'spiking' procedure of immediately mixing the ^3He with the unknown ^4He gas sample was not followed because the ^3He spikes also contained $\sim 1 \times 10^{10}$ atoms of ^4He . A nearly constant mass discrimination of the mass spectrometer was repeatedly measured during each series of runs by analysing precisely known mixtures of ^3He and ^4He introduced from other vacuum enclosures.

The results of helium analyses of samples and controls are given in Table 1. The measurements were complicated slightly by a nearly constant small residual helium background displayed by the mass spectrometer system, resulting from a steady permeation of helium through glass components and from the desorption of helium from previous larger gas samples. Although this background changed slightly from day to day, it varied little within any given day as shown in column 5 of Table 1. The uncertainty in the measurement of this background ($\sim \pm 2 \times 10^8$ atoms ^4He) was the major source of uncertainty in the results.

The sample vacuum enclosure has mountings for four tungsten coils so that a combination of two samples and two controls could be vaporised in 2 h. Comparison of the data from the control samples (column 4) with background values (column 5) shows that no perceptible additional helium was released either by the control samples or by the heating of the tungsten coils. The results in column 3 for seven of the ten stratospheric particles, however, are clearly outside the range of the system background. The net ^4He released by these particles (column 6) ranged from a barely perceptible 4×10^8 atoms for sample SP-3 to a sizeable 2.7×10^{10} atoms for SP-14.

Measurements made of the ^3He released by the particles and controls, not listed in Table 1, were subject to the memory effects of the ^3He spikes. Consequently, net measured ^3He values of 1.9×10^9 and 4×10^8 atoms, respectively from samples SP-2 and SP-15 are not considered to be real. The other samples showed no measurable ^3He above background levels.

The final column in Table 1 lists helium concentrations ranging from 0.002 to 0.25 cm^3 (STP) g^{-1} . Such high concentrations confirm that the particles were extraterrestrial,

and that some or all of them were exposed to solar wind for at least 10–100 yr. Since solar wind ions are implanted only to depths of ~ 500 Å, the measurements also indicate that the particles existed as small particles in space and were not produced in the atmosphere by fragmentation of larger meteoroids.

The possibility that the observed helium could instead have been the product of decaying uranium seems remote. Production of the high level of helium found in sample SP-14, for example, would require a uranium concentration of 200 weight parts per million (w.p.p.m.) and a decay time of 4.6×10^9 yr. Although phosphate and zircons, sometimes found as inclusions in meteorites, can contain up to ~ 20 w.p.p.m. uranium, non-destructive X-ray analyses of these particles have shown their compositions closely to resemble chondritic meteorites which generally have uranium concentrations three orders of magnitude lower than the latter figure.

The compositional similarity of all ten particles, together with the observed presence of helium in seven of them suggests that all ten are of extraterrestrial origin. The absence of observable helium in three of the particles and the widely varying concentrations observed in the others can be ascribed to varying amounts of heating during entry into the atmosphere. It is estimated that with typical entry parameters a 10- μm particle will experience a thermal pulse of 500 °C or more and that this pulse will last for several seconds. The particles in this study which showed high helium concentrations after entry into the atmosphere are true micrometeorites in the sense that they have not experienced any substantial heating. Micrometeorites thus join meteorites and lunar samples as sources of extraterrestrial materials which can be analysed in the laboratory for clues to early Solar System processes. Because micrometeorites have probably cometary origin¹⁰ they are potentially a valuable source of primitive Solar System matter.

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Pulsating aurora induced by upper atmospheric barium releases

AN effect which appeared to be pulsating aurora was observed to be associated with explosive releases of barium vapour near 250 km altitude, but only when the explosions were in the path of precipitating electrons associated with the visible aurora.

The primary purpose of this series of rocket experiments, launched from Poker Flat, Alaska ($65^{\circ} 77.5' \text{ N lat.}, 147^{\circ} 28.91' \text{ W long.}$) during March 1976, was the measurement of the magnitude and direction of electric fields and neutral winds in the auroral atmosphere¹. Each explosive charge was a standard 1.5 kg thermite mixture of Ba and CuO with an excess of Ba metal which was vaporised and dispersed by the thermite explosion². Traces of Sr, Na and Li were added to some of the charges in varying amounts, and an extensive set of spectrophotometric observations from the ground was planned to determine the effectiveness of each of these luminous, un-ionised species in tracing the upper atmospheric, neutral wind.

The two flights of interest here were made in the morning twilight on 27 and 28 March 1976, at about 1250 and 1300 UT, respectively. The approximate trajectory of the rockets is shown in Fig. 1 along with the location of the four releases and the ground stations. One difference between the two flights is that perigee on the 27 March flight was approximately 50 km higher than that for the 28 March flight.

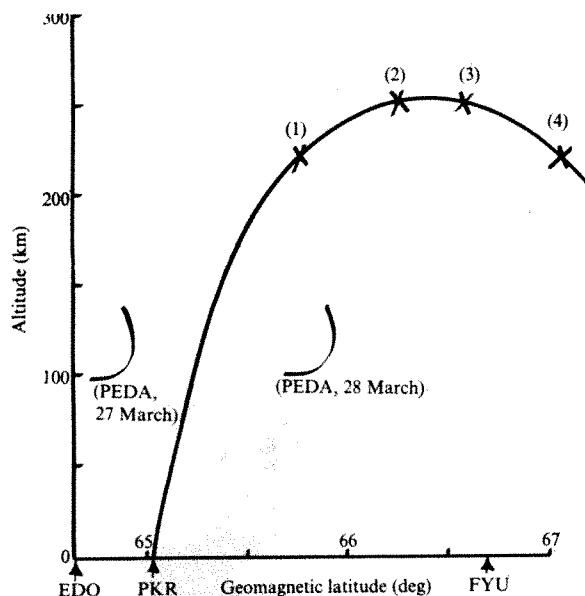


Fig. 1 An elevation view of the rocket trajectory on 28 March 1976, in the geomagnetic meridian ($\sim 30^{\circ} \text{E}$ of true N) through the Poker Flat Research Range. Shown also are the approximate positions of the observing sites (Ester Dome: EDO, Poker Flat: PKR, Fort Yukon: FYU), the barium releases and the natural aurora. Figures in parentheses are release nos. PEDA, poleward edge of diffuse aurora.

Spectrophotometric observations were made from two locations, Ester Dome ($64^{\circ} 52.8' \text{ N lat.}, 148^{\circ} 3.4' \text{ W long.}$) and Poker Flat. Emission spectra were obtained for all the releases using a television spectrograph at Ester Dome. With this instrument, spectra between 4,000 and 8,000 Å at 40 Å resolution of chemical release clouds as well as intense aurorae are recorded on video tape at a rate of 30 frames per second. Focusing the sky on the entrance slit gives an angular spatial coverage of 8° (resolution: $< 0.5^{\circ}$)

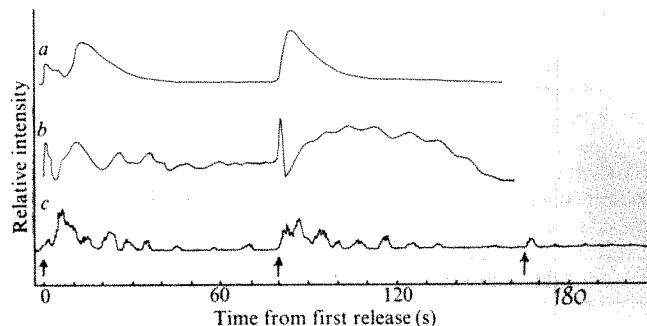


Fig. 2 Observations of two atmospheric emissions and one neutral Ba emission associated with upper atmospheric Ba releases made on 28 March 1976, from Poker Flat, Alaska. The times of three of the four releases are indicated by arrows. The fourth release showed neither enhancement nor pulsation of the atmospheric emission. a, 6,110 Å BaI, TV spectrograph; b, 5,577 Å OI, TV spectrograph; c, 4,278 Å N_2^+ , photometer.

along the spectral line. At Poker Flat an optical interference filter photometer with an 8° field-of-view was used to observe 4,278 Å (N_2^+ ING) emission in each release and record the amount of ionisation of atmospheric constituents. Numerous other instruments making support observations included two wavelength-scanning Ebert-Fastie spectroimeters at Poker Flat, the Chatanika Incoherent Scatter Radar, and all-sky cameras, riometers and meridian scanning photometers at Poker Flat and Fort Yukon ($63^{\circ} 34' \text{ N lat.}, 145^{\circ} 17' \text{ W long.}$).

The morphology of the aurora on the occasion of both rocket flights was quite similar and characteristic of early morning aurorae in the Alaskan sector. Towards the equator was a large area of pulsating aurora and auroral glow. This area was terminated on the poleward side by a series of closely-spaced arcs appearing as striations in an E-W direction. Arc segments peeled off this boundary towards the pole with an overall eastward motion, forming so-called 'omega bands'. All of these features are part of what is referred to as the 'diffuse' aurora because of its apparent lack of structure to satellite-borne cameras³. The poleward, 'discrete' aurora consisted of weak, rayed arcs and arc segments. The location of the boundary between the diffuse and discrete auroral regions seemed to be relevant to the occurrence of the pulsating aurora effect which is the subject of this report. This location is shown schematically in Fig. 1.

On 28 March 1976, a distinct cloud of 5,577 Å [OI] emission was detected on the TV spectrograph located just below the bright Ba cloud on releases nos. 1 and 2, but not on nos 3 and 0. The photometer operated at Poker Flat observed 4,278 Å (N_2^+ ING) emission associated with each release as shown in Fig. 2, together with the 5,577 Å data from the TV spectrograph. It is apparent that there is an enhancement of both emissions associated with the first two bursts and that these emissions pulsate with roughly a 10 s period for approximately 60–100 s following the burst. The 4,278 Å pulsations were of the order of 100 rayleighs against a background of 1 kR. This would be produced by a flux of approximately $5 \times 10^8 \text{ cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$ of 500 eV electrons which would be deposited near the observed altitude of 200 km. Also from theory⁴, the corresponding intensity of 5,577 Å OI emission should be approximately 1 kR which is in reasonable agreement with TV spectrograph data, although that instrument is not calibrated on an absolute scale. No 5,577 Å OI emission was detected on March 1976, when all the clouds were poleward of the diffuse auroral boundary. Thus, because the enhancements occurred only when the chemical releases were above the diffuse aurora (or nearly so), it is tempting to assume that the presence of the diffuse aurora gives an indication of the conditions necessary to produce the pulsations.

Other geophysical effects associated with the early morning diffuse aurora were considerable absorption of galactic radio noise indicating D region ionisation produced by energetic auroral primary electrons, and electron densities in the E region $>10^5 \text{ cm}^{-3}$ observed by the Chatanika Radar. Table 1 summarises the observations from Poker Flat, indicating differences in the release environment which may be related to the production of the induced pulsations.

Table 1 Summary of observations during Ba releases

	27 March 1976 ~64.5° inv. lat.	28 March 1976 ~66° inv. lat.
Northern border of diffuse aurora		
D region radio wave absorption	Small	Large
E region electron density	$<10^4 \text{ cm}^{-3}$	$>10^5 \text{ cm}^{-3}$
F region electron density	$<10^4 \text{ cm}^{-3}$	$<10^4 \text{ cm}^{-3}$
Ionospheric electric fields	Weak, variable direction	Medium N-S directed, horizontal
Discrete aurora	Weak-rayed bands	None visible

The steady westward drift of ion clouds on 28 March indicated the existence of a N-S-directed, horizontal E field characteristic of early morning aurora. On 27 March the ion clouds striated and merged with the weak, discrete auroral bands indicating only variable, local E fields.

The TV spectrograph provides both spatial and spectral information on the development of the luminous cloud. The radius of the cloud was determined separately for the Li and Ba debris for release no. 3 on 27 March 1976 and both the Ba and Li clouds were observed to oscillate with a frequency near 1 Hz during the first 15 s after release. These high frequencies were not observed in the clouds which produced the auroral pulsations. Oscillations of longer periods (10 s) would be difficult to observe over the limited lifetime of the induced auroral pulsations. Thus, the observed high frequency oscillations may indicate the presence of lower frequency modes which could be intimately connected with the mechanisms producing the induced auroral pulsations.

Previous observations of enhanced auroral emissions and E region electron densities associated with F region Ba releases were made of the E region below the cloud by Stoffregen^{5,6} and although they showed the same overall time variation, there was no definite evidence of pulsations. In the case reported here, the diffuse aurora in the E region masked any enhancement which may have occurred and the observed pulsations were located just below the Ba cloud (~200 km altitude). Because the period of the pulsations was in the Alfvén or magnetoacoustic wave domain (10 s) and approximately that which is most often observed in the natural aurora at this latitude, attempts will be made in the near future to repeat the experiment in hopes of clarifying the mechanism for the natural production of pulsating aurora.

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Configuration of the magnetic field and reconstruction of Pangaea in the Permian period

THE virtual geomagnetic poles of Laurasia and Gondwanaland in the Carboniferous and Permian periods diverge significantly when these continents are reassembled according to the fit calculated by Bullard *et al.*¹. Two interpretations have been offered: Briden *et al.*² explain these divergences by a magnetic field configuration very different from that of a geocentric axial dipole; Irving³ (and private communication), Van der Voo and French⁴ suggest a different reconstruction and it is shown here that these two interpretations are not incompatible and that the first may help the second.

I have developed a program which allows a spherical harmonic analysis of the palaeomagnetic field⁵ by a method similar to Kono's⁶. The magnetic field F_i at the point of colatitude θ_i and longitude ϕ_i is given by the equation

$$F_i = \sum G_j f_{ij}$$

where G_j is the expansion coefficient in spherical harmonics, and f_{ij} the vector whose components are trigonometric polynomial functions of θ_i and ϕ_i (notation used by Creer *et al.*⁷). A_i is the measured palaeomagnetic direction of unit length at the same point.

We need to minimise the following residual: $\Sigma(e_i)^2 = \Sigma(F_i/|F_i| - A_i)^2$ where $|F_i|$ is the modulus of F_i calculated starting from a first expansion. So a system of linear equations

Fig. 1 Proposed reconstruction. Transverse Mercator projection. The symbols indicate the location of the virtual geomagnetic poles that were used. Parallels and meridians are drawn every 30°.

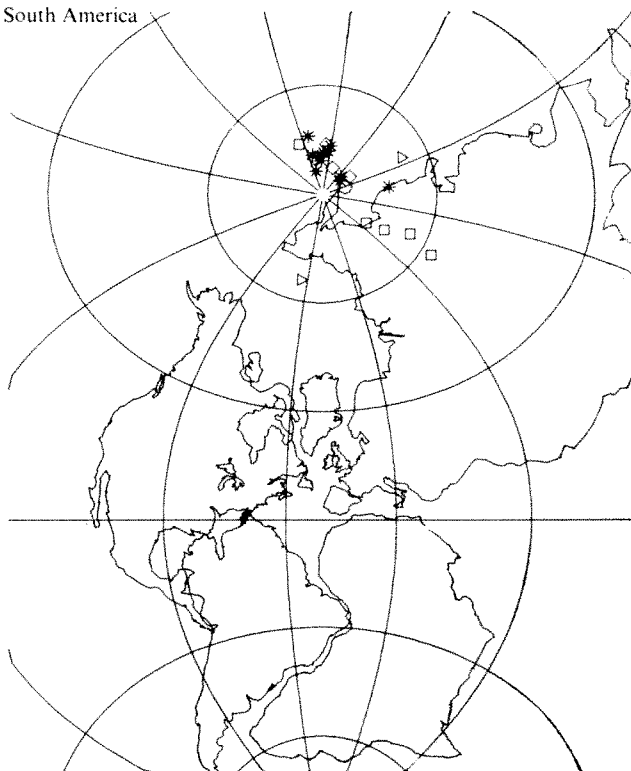
○ Morocco

▷ Africa

* Europe

◇ North America

□ South America



with unknowns G_j is given, the system solved and new values calculated for $|F_i|$, and the calculation repeated until the solution is stabilised.

The first eight terms of the expansion allow calculation of the orientation of the dipole (geomagnetic poles) and the position of its centre (see formulae in Creer *et al.*⁷). I have applied this method to the problem of the magnetic field in the Permian and I have grouped the palaeomagnetic results for neighbouring sections and then analysed the data thus obtained separately for the two aggregates Africa-South America (8 groups) and Europe-North America (14 groups).

If these continents are reassembled according to Bullard's fit, with Africa in its present position, the results are as follows:

	geomagnetic axis		centre of the offset dipole	
			distance	direction
Laurasia	46.5°N	227°E	1170 km	33°S 205°E
Gondwana	29°N	238°E	780 km	29°S 183°E

In both cases the angle between the direction of the geomagnetic axis and the direction of the centre of the offset dipole is about 80°. This configuration is quite different from the mean axial field observed in the Tertiary, but is similar to the present-day field. This lateral offset of the dipole allows us to resolve the problem of relative longitudes of the two supercontinents.

I have first superposed both geomagnetic poles and then rotated one continent around this common axis until the directions in which the dipoles are offset are coincident. A relative rotation of Europe and North America of -35° around a pole situated at 22°N and 25°E (with respect to Africa) is a satisfactory solution and the corresponding reconstruction is shown in Fig. 1, with the common geomagnetic axis placed on the geographic pole. This reconstruction is close to that proposed by Irving³ for the same period, but with different premises.

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Isotopic evidence for dramatic climatic changes in East Africa during the Pleistocene

RAINFALL decreased dramatically in the Lake Turkana region 1.8-2.0 Myr ago and in the Olduvai Gorge region 0.5-0.6 Myr ago. This is documented by a major increase in the $\delta^{18}\text{O}$ values of pedogenic and groundwater carbonates at these times. The data suggest that meteoric water of the earlier, more humid climate was 2-4 per mil lower in ^{18}O content than modern waters of these regions.

A terrestrial stratigraphic record covering a large portion of the Pleistocene is found in several sedimentary basins in the eastern Rift zone of East Africa. We have examined the stable isotope variations through the Pleistocene at Olduvai Gorge in northern Tanzania and in the sediments surrounding Lake Turkana in northern Kenya (Fig. 1). Thirty-three samples of carbonates were analysed as were several samples of modern rain and lake waters from the two areas. Oxygen isotope compositions of carbonates are controlled by the

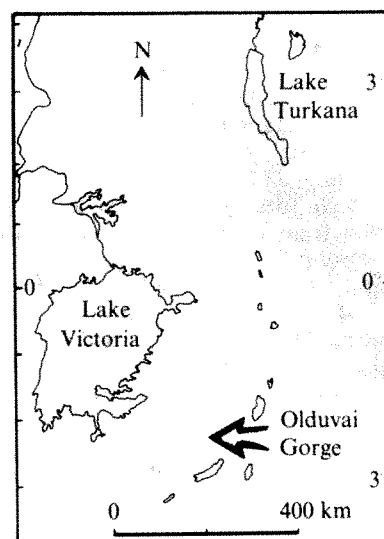
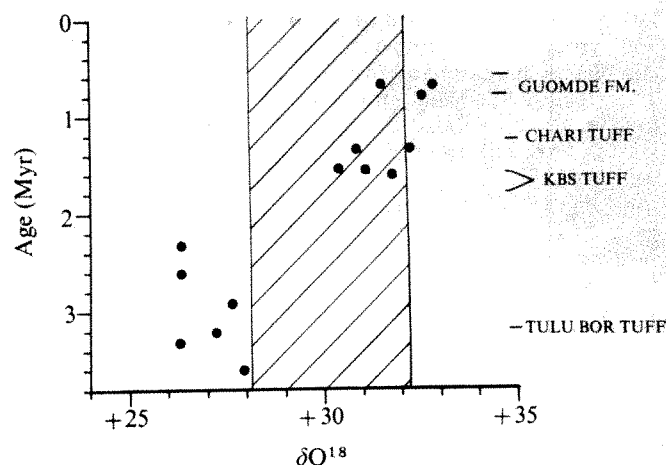


Fig. 1 Location map.

oxygen isotope compositions of the formation waters and by temperature¹. The oxygen isotope compositions of meteoric waters, in turn, are controlled by climatic factors^{2,3}. Consequently, isotopic analyses of groundwater carbonates provide excellent indicators of climatic conditions prevailing at their time of formation.

Three types of carbonates were analysed: pedogenic calcretes, pedogenic nodules, and groundwater nodules from lacustrine clays. The calcretes are impervious and represent sealed environments. This is documented by ^{14}C dating of 17 samples of upper Pleistocene and Holocene calcrete from Olduvai Gorge⁴. The dates fit with those determined on organic material from the same sequence and there is no overlap between the different stratigraphic levels. Further details of age relationships at Olduvai Gorge are documented by Hay⁴ and at Lake Turkana by Curtis *et al.*⁵ and Fitch and Miller⁶. Although some controversy still exists as to the age of the KBS Tuff at Lake Turkana, this tuff marks the climatic change discussed later. The Olduvai calcretes comprise both massive and laminated varieties, both of which were formed by meteoric water percolating downward through the soil column⁷. The

Fig. 2 A plot of $\delta^{18}\text{O}$ values of carbonates against age at Lake Turkana. The hatched area represents the isotopic compositions of theoretical carbonates in isotopic equilibrium with waters that have the range of $\delta^{18}\text{O}$ values for rain measured at Lake Turkana and Olduvai Gorge. The values were calculated for 25 °C using the equation of O'Neil *et al.*⁸. Stratigraphical relations are shown at the right.



lacustrine nodules appear relatively impervious and presumably also represent sealed environments; also, groundwater nodules occur in relatively impermeable clays.

In the section at Lake Turkana, samples older than 1.8 Myr have $\delta^{18}\text{O}$ values in the range +26.3 to +27.9, whereas those younger are relatively enriched with a $\delta^{18}\text{O}$ range of +30.4 to +33.0 (Fig. 2). At Olduvai Gorge, the Masek Beds which formed 0.4 to 0.6 Myr ago⁴ mark a similar transition from light to heavy carbonates: Beds I, II, III and IV have $\delta^{18}\text{O}$ values of +24.1 to +27.1 and the Ndutu, Naisiusiu and Holocene sediments have $\delta^{18}\text{O}$ values of +30.9 to +31.8 (Fig. 3). At Olduvai, a dramatic event took place during Masek time and perhaps a minor one occurred at the end of Bed I (1.7 Myr ago⁴). A general increase in $\delta^{13}\text{C}$ with $\delta^{18}\text{O}$ was also observed in both areas.

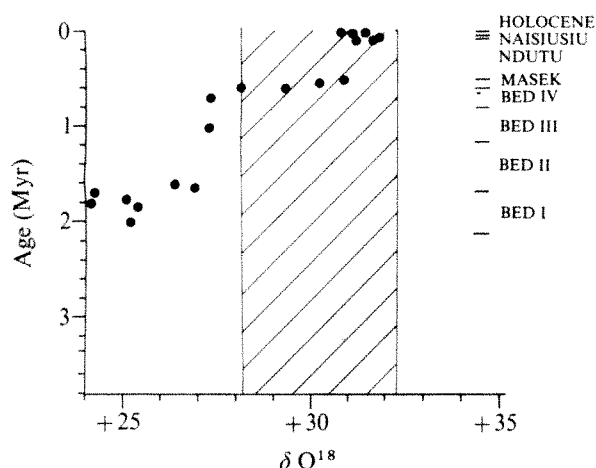


Fig. 3 As for Fig. 2, for Olduvai Gorge.

Craig⁹ has shown that East African waters are abnormally rich in the heavy isotopes of oxygen and hydrogen. The isotopic data do not fall on a line characteristic of world-wide meteoric water ($\delta D = 8\delta^{18}\text{O} + 10$) but are instead on a line characteristic of evaporating waters ($\delta D = 5\delta^{18}\text{O} + 10$). This is a result of the fact that the rain partially evaporates as it falls through the dry East African atmosphere. Analyses of five rain waters from Olduvai Gorge and Lake Turkana ranged in δD from +4.9 to +26.0 and in $\delta^{18}\text{O}$ from -0.4 to +3.5. The δD and $\delta^{18}\text{O}$ values of Lake Turkana and Lake Ndutu (west of Olduvai Gorge) are +37.1 and +5.84, and +41.7 and 6.7, respectively. These measurements concur with Craig's observations of unusually heavy waters in East Africa. Rain water has the lightest isotopic values of all the waters possibly involved in carbonate formation in this area. Modern waters from East Africa could not produce the relatively low $\delta^{18}\text{O}$ values observed in the older sediments unless soil and groundwater temperatures were 35–45 °C. This is completely unreasonable on the basis of faunal evidence. Therefore the soil and groundwaters from which the older carbonates precipitated originated from rainfall that was at least 2–4 per mil lower in ^{18}O content than found in modern waters of the region. This is favoured by lower evaporation rates and therefore implies that there was more rain in the past. Moreover, increased humidity might be expected to result in a decrease in temperature, which would cause a further decrease in $\delta^{18}\text{O}$ values of rainfall and groundwater. These results point to a drastic decrease in rainfall about 1.8–2.0 Myr ago at Lake Turkana and 0.5–0.6 Myr ago at Olduvai Gorge.

The Olduvai Beds contain faunal, lithologic and mineralogic evidence of increasing aridity from Bed I with a semi-arid climate, to the Masek Beds with an arid climate similar to that prevailing today. Bed I contains faunal

elements (e.g., the urocyclid slug) indicating moister conditions than any later deposit at Olduvai; the Masek Beds have forms characteristic of drier climates (e.g., the land snail *Limicolaria*)⁴. Extensive deposits of wind-worked tuffs occur from the Masek Beds to the present indicating drier conditions. The minerals formed by weathering in the Masek Beds (illite, zeolites, dawsonite) also indicate a climate drier than recorded previously at Olduvai Gorge⁴.

The sediments surrounding Lake Turkana also show evidence for a climatic change that coincides with the isotopic anomaly. Volcanic ash below the KBS Tuff (1.8 Myr old⁵) is altered to montmorillonite whereas that above it is altered to montmorillinite, chabazite and clinoptilolite. The shoreline of the lake that is older than 1.8 Myr was stable indicating that it probably was an open basin, whereas the fluctuating nature of the post-KBS shoreline and the presence of zeolites indicates that the lake periodically fell below overflow level.

The causes of the change in rainfall patterns are not clear from the study of these two areas. The climate of East Africa today is controlled by the intertropical convergence zone and the monsoons from the Indian Ocean^{10,11}. Any change in the intertropical convergence patterns, the relative positions and amount of highlands and their rain shadow effect, or in large bodies of water such as Lake Victoria, could have drastic effects on the amount of rainfall.

We are now determining the hydrogen and oxygen isotope compositions of hydrous authigenic minerals in an attempt to characterise more closely the isotopic composition of palaeometeoric waters in the region.

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Location and age of Mesolithic coastal occupation sites on Oronsay, Inner Hebrides

THE existence of at least five Mesolithic occupation sites on the island of Oronsay, Inner Hebrides, has now been established (Fig. 1). Preliminary results of recent excavation of two of the sites were reported in 1971 (ref. 1). Since then further excavations have been made and the locations and ages of the sites in relation to the position of the shore line of the sea at the maximum of the Holocene marine transgression have been investigated. The results obtained suggest that the ages of some of the sites in relation to the former shore line cannot be established solely on stratigraphical

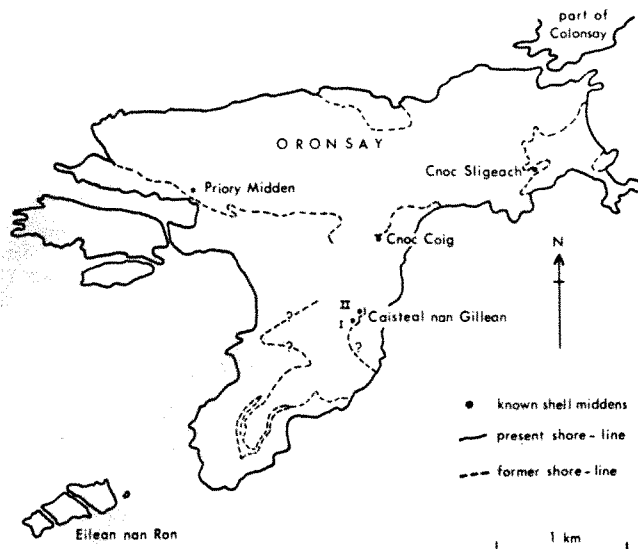


Fig. 1 Map of the Scottish Hebridean island of Oronsay (56° N, 6° W), showing the locations of known Mesolithic occupation sites and the position of the visible parts of the former shoreline mentioned in the text.

and altitudinal evidence but require supplementary information based on radiocarbon dating. It will be some time before the full details will be published, and so a short account of the results so far obtained is presented now.

The position of the former marine shore line differs markedly from that of the present coastline of the island of Oronsay (Fig. 1). The old shore line is obscured in places by blown sand, especially in the area between Caisteal nan Gillean and Cnoc Coig. It is possible, however, that at the maximum of the Holocene marine transgression Oronsay consisted of two main islands: a larger area of relatively high ground and a smaller low rocky area somewhat similar in aspect to the present Eilean nan Ron (Fig. 1) which supports a seal colony and is surrounded by limpet-inhabited rock skerries exposed at low tide. The islands may have been connected with each other at low tide as Oronsay is with Colonsay at present.

Measurement of high water level and low water level of spring tides in the summers of 1973, 1974, 1975 and 1976 at a number of stations along the eastern and western shores of Oronsay showed that present (summer) maximal tidal range varies between ~ 3.5 and 4.3 m, spring tidal range for practical purposes being 4.0 m, from -2.0 m to $+2.0$ m in relation to Ordnance Datum, Newlyn². If the tidal range around Oronsay at the time of the maximum of the Holocene marine transgression was approximately the same as at present, the contemporaneous Mean Tide Level (which is approximately the same as Mean Sea Level but is not precisely equal to it³) was $\sim +7$ m Ordnance Datum (OD) and the intertidal zone of that time was the area now standing at $\sim +5$ to $+9$ m OD. Measurements of the altitude of cobble debris deposited by modern severe storms (those of January and February 1974) suggests that along most of the coast of Oronsay similar storms at the maximum stand of the Holocene marine transgression may have thrown debris to locations that now occur up to $+11$ m OD. Storm debris might be expected to accumulate to somewhat higher levels than this on the crests of the cobble ridges that fringe the exposed southern peninsula of the former coast. The highest points on the crests of these ridges in fact occur at $\sim +11.4$ m OD (western ridge), $\sim +12.7$ m OD (southern ridge) and $\sim +13.1$ m OD (eastern ridge).

Three of the known Mesolithic occupation sites are located in positions that are above the highest probable high

water levels of ordinary spring tides and severe storms of the Holocene marine maximum. Occupation material of the Priors Midden occurs at levels between $\sim +12.8$ and $+14.3$ m OD. In the vicinity of the site the highest part of a (probably contemporaneous) marine cobble ridge occurs at $\sim +9.0$ m OD; other possibly contemporaneous beach deposits occur up to $\sim +11.9$ m OD. At Caisteal nan Gillean I occupation material is located on the crest of a sand mound between $\sim +16.0$ to $+17.3$ m OD and on the perimeter of the same mound at $\sim +11.1$ m OD. On the adjacent site of Caisteal nan Gillean II occupation material occurs at $\sim +12.5$ to $+15.5$ m OD, possibly fractionally lower, but certainly higher than $+11.0$ m OD, the probable upper limit reached by waters of severe storms when the Holocene sea stood at its highest level.

The two remaining known occupation sites, Cnoc Coig and Cnoc Sligeach, are located closer to the former shore line, both in altitude and position, than any of the other sites. Cnoc Coig is a low mound of (mainly) invertebrate shell debris, its crest at $\sim +10.2$ m OD, and the debris, up to ~ 1 m thick, rests on sand (possibly blown or beach sand) that in turn rests on solid rock. The shell mound is bordered

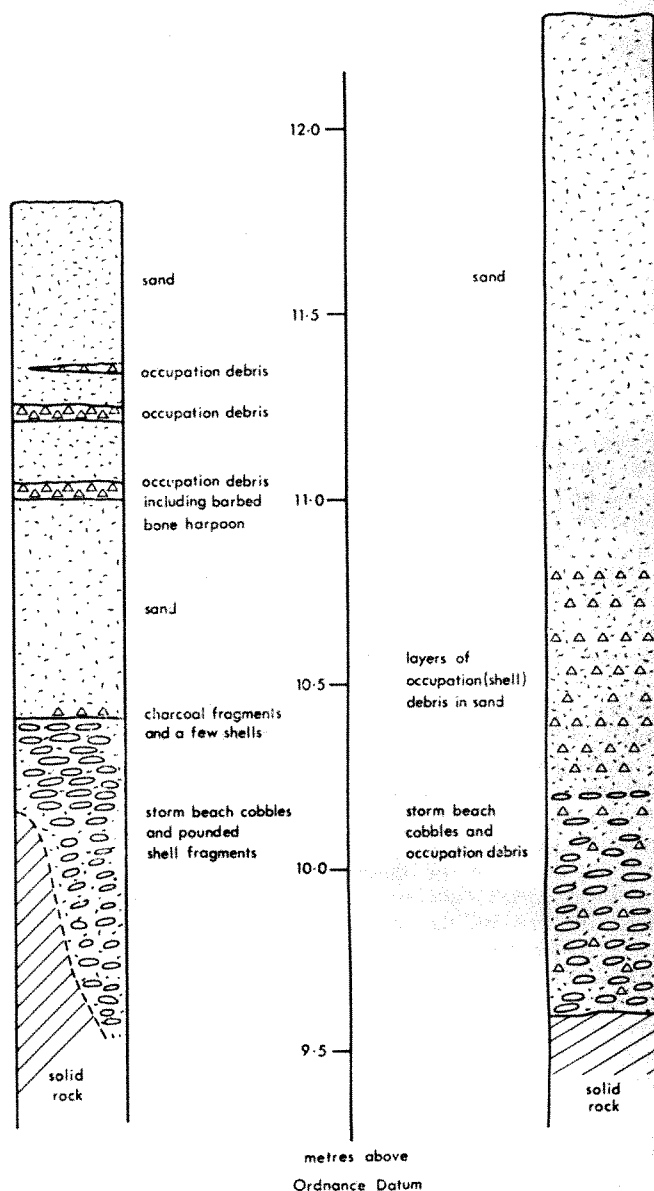


Fig. 2 Simplified vertical sections of recent excavations 30 m to the south of the crest of Cnoc Sligeach (left) and of excavations in 1913 by W. H. Bishop 20 m to the south-east of the crest of Cnoc Sligeach (right).

Table 1 Radiocarbon age determinations at Mesolithic occupation sites on Oronsay

Site	Nature of material dated	Position of sample within occupation debris	Lab no.	Age of sample in radiocarbon years (b.p.)
Caisteal nan Gilleann II	Wood charcoal { Humate abstract	Upper half of midden	Birm-346	4,920 ± 400
	Wood charcoal { After alkali pretreatment		Birm-347	5,150 ± 380
	Shells of <i>Patella vulgata</i>	Base of midden	Birm-348	5,450 ± 140
				5,570 ± 140 (outer)
Cnoc Coig	Wood charcoal	Base of midden	Birm-348	5,720 ± 140 (middle)
	Wood charcoal	Base of midden		5,850 ± 310 (inner)
	Wood charcoal	Base of midden	Q-1355	5,460 ± 65
	Wood charcoal	Base of midden	Q-1352	5,430 ± 130
Cnoc Sligeach	Wood charcoal	Upper part of midden	Q-1351	5,495 ± 75
	Wood charcoal	Upper part of midden	Q-1354	5,535 ± 140
	Wood charcoal	Base of midden	Q-1353	5,645 ± 80
	Valve of <i>Ostrea</i> sp.	Base of midden	GX-1903	5,015 ± 210
	Animal bone fragments (collagen)	Positions not known	GX-1904	5,755 ± 180
	Wood charcoal	Upper part of midden (composite sample)	BM-670	5,426 ± 159

on the north by a low rock exposure that was immediately adjacent to, if it did not itself form, the margin of the shore at the maximum of the Holocene marine transgression. The lowest level at which occupation material occurs at this site is ~ +9.0 m OD, approximately the same altitude as the estimated normal high water level on Oronsay at the maximum of the Holocene marine transgression. On this basis it is arguable that the Cnoc Coig site was occupied at the time of the marine maximum. Other evidence, however, argues strongly against this: the site is located on the seaward side of the rock exposure that (conjecturally) formed the contemporaneous shore line; fifty metres to the north of the site Holocene storm debris is present at levels up to ~ +9.5 m OD, but at the site itself no traces of storm debris in association with occupation material were found in the course of recent excavations. It may be concluded that the site certainly was not occupied during storms at the time of the marine maximum, and it is probable that the site was never occupied until after the sea had receded from its maximal position.

Occupation material at Cnoc Sligeach occurs near the crest of the mound at ~ +16.9 m OD, on the sides of the mound and around several centres located on the periphery of the mound. The lowest level at which substantial quantities of occupation material were found recently at this site was ~ +10.4 m OD, the material concerned (wood charcoal in association with shells of *Patella* sp. and *Littorina littorea*) occurring on the southern periphery of the mound and resting directly on storm-beach cobbles. In addition, a few small, diffuse, dark-brown or black patches, possibly of charcoal, were found sporadically distributed within the mixture of storm beach cobbles and pounded fragments of *Patella* sp. that extended downwards to solid rock at the same location (Fig. 2). The evidence is comparable with, but not precisely the same as, that described by Bishop⁴ whose extensive excavations on the south-eastern flank of the mound in 1913 demonstrated that the maximum altitude at which marine cobbles were deposited there was ~ +10.2 m OD. Bishop also noted that, at the site of his investigation, 'throughout the entire depth of this beach were found the same implements and remains as were found in the overlying shell refuse' (ref. 4). In Fig. 2 the vertical profile revealed by Bishop's excavations is compared with that of the recent investigations. It should be noted also that to the south-west of Cnoc Sligeach the storm-beach debris forms a narrow bar linking two rock knobs. Here the crest of the cobble and boulder debris reaches altitudes up to ~ +11.6 m OD. Collectively the evidence from locations on the south-western, the southern and the south-eastern periphery of Cnoc Sligeach suggests that the site was first occupied about the time of the Holocene marine maximum

or at what was only a short time after the maximum, when severe storms still were building cobble ridges up to levels of ~ +10 to +11 m OD.

The data presented above demonstrate that it is not possible to determine the age relationships between three of the known occupation sites (the Priory Midden and Caisteal nan Gilleann I and II) and the shore line at the (local) maximum of the Holocene marine transgression solely on the basis of stratigraphical and altitudinal evidence. They also suggest that the Cnoc Sligeach site may have been occupied slightly earlier than the Cnoc Coig site. Clearly another basis must be used to determine the age relationships among the five sites themselves and between each individual site and the shore line of the Holocene marine maximum.

Radiocarbon age determinations on occupation material are available from three of the sites (Table 1). The data point to the approximate contemporaneity of the occupation sites at Caisteal nan Gilleann II and Cnoc Coig; statistically no age difference can be established between Birm-347 on the one hand and Q-1353 and Q-1354 on the other (test used: $\Delta A \leq 2\sqrt{(\sigma A_1^2 + \sigma A_2^2)}$). The place of the Cnoc Sligeach site in the occupation chronology is uncertain as yet. This is because one of the three radiocarbon-dated samples was composite in nature (BM-670) and the exact provenances of the other two are not known (GX-1903 and GX-1904). Recent investigations yielded suitable material for radiocarbon dating from accurately identified positions at the Priory Midden, Caisteal nan Gilleann I and Cnoc Sligeach. When the samples have been dated it should be possible to establish the chronology of the sites in relation to each other and the former shore line by combining the evidence of radiocarbon dating with that provided by stratigraphical relationships and altitudinal positions.

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Spontaneous formation of bubbles in gas-supersaturated water

SPONTANEOUS nucleation of bubbles may occur in water subjected to heating¹⁻³, tensile stress⁴⁻⁶, or gas-supersaturation⁷⁻¹². Large discrepancies exist between the threshold nucleation conditions predicted theoretically and those indicated by empirical observations¹³. The theoretical approaches have been hampered by inadequate understanding of many of the submicroscopic and molecular properties of liquids, and experimental obstacles have included the always present and difficult to control interference from pre-existing nuclei. These problems have been most pronounced for nucleation induced by dissolved gas; indeed, nucleation thresholds for any gas/liquid system are unknown due to a lack of relevant empirical data, and no quantitative theories seem to exist.

Recent investigations of bubble formation at glass-water interfaces have indicated some minimum nucleation threshold limits for various gases in bulk water^{11,12}. More direct determinations of threshold values, obtained by cinemicrography, are reported here. The results raise some questions about the conventionally accepted relationship between surface tension and pressure in bubbles and the submicroscopic behaviour of water.

In the method used, water equilibrated at high argon gas pressures was decompressed to ambient, atmospheric pressure while it was observed for bubbles. The apparatus (Fig. 1) consisted of a 25 ml chamber for equilibration, A, connected to a 0.5 ml cuvette chamber, B, formed between

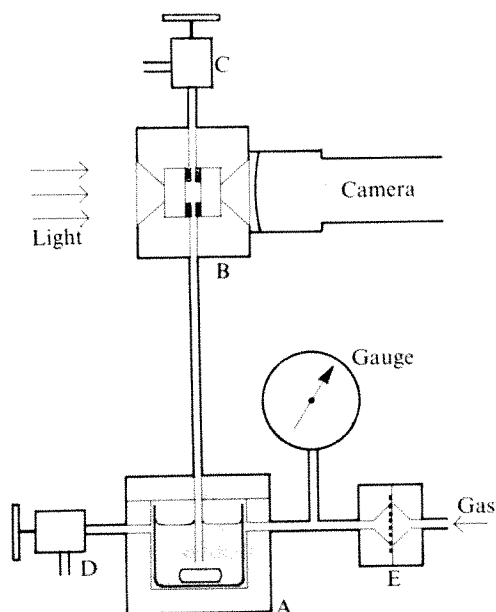


Fig. 1 Schematic diagram of the apparatus which consists of an equilibration chamber A containing a Pyrex cup with a magnetic stirring bar, a cuvette chamber for observations B, valves C and D and Millipore filter unit E. All parts in contact with water were made of stainless steel. A quartz lamp with a light-diffusing, heat-absorbing filter provided illumination.

optical type Pyrex windows (0.64 cm thick, 2.5 cm in diameter) separated by a flat stainless steel ring (0.30 cm thick, 1.40 cm inner diameter). The water in the transilluminated cuvette chamber could be photographed with a 16 mm, 64 frames s^{-1} , movie camera, equipped with an inverted lens of 2.5 cm focal length and an aperture of $f/11$; the lens to film distance was approximately 18 cm. The full frame coverage was about 0.14×0.10 cm, with about 0.02 cm usable depth of field. Before use, all important parts of the apparatus were dichromate-sulphuric acid cleaned and well rinsed. Double glass-distilled water was filtered through a

Millipore filter, pore size $0.45 \mu m$, before equilibration with argon of 99.99% purity. The gas was admitted to the chamber through the same type of filter. Some comparison experiments were performed without filtration.

When gas pressure is applied initially to the system, the water, still with little gas in it, is hydrostatically pressurised; this forces many potential pre-existing gas nuclei into solution. After equilibration at argon pressures ranging from 140 to 202 atm, the water was flushed under pressure through the cuvette chamber by slow and repeated copious bleedings at the valve on the top of the chamber outlet tube, with the assembly in the upright position as shown. The assembly was then turned to the horizontal position, and placed in a predetermined location relative to the camera lens, which had been focused on the nearest inner glass surface. Decompression to ambient pressure was performed over a period of 2-3 s with the camera running. A total of 27 such experiments were performed at room temperature ($20-22^\circ C$).

The films were examined frame by frame with $\times 20$ magnification. Bubbles could be detected at $5 \mu m$ diameter; those which arose in the water usually could be distinguished easily from those in focus and attached to the glass surface, as the former would usually be out of focus to varying degrees and would show some motion in unison due to a slight inevitable movement of the water caused by the volume expansion of the bubbles.

With the water saturated with argon at 155 atm and lower pressures, bubbles formed only occasionally within the relatively narrow field of the camera, and then only on the glass surface and at decompression pressures close to ambient value. At 158 atm saturation, some bubbles consistently formed on the glass surface. A few also could be observed in the bulk water, but there was some uncertainty as to whether they originated there. With progressively higher saturations the number of bubbles increased, with the largest increase by far occurring in the bulk water, and the bubbles started to form at higher decompression pressures, the higher the saturation. There was a notable synchronous appearance and even distribution of the bubbles in the water, with no coalescence in the early stages. At 165 atm, substantial numbers of bubbles (20-40 per μl) quite clearly developed in the bulk water. This was even more evident during decompression from higher saturations: at 177 atm, the majority of the bubbles (several hundred per μl) appeared in the water and, at 202 atm, at the time bubbles massively occurred in the water (several thousand per μl), relatively few bubbles could be observed on the glass surface.

Because of their presence in such large numbers, their synchronised appearance, and their even distribution, the bubbles observed in the water in general must be nucleated by a spontaneous, homogeneous process involving only the water and the gas dissolved in it, rather than arising from preformed gaseous nuclei in suspended motes or the interface of the motes *per se*. This is supported by the lack of any obvious effect when micropore filtering was eliminated, and also by the lack of any effect on the general cavitation behaviour of water after 1,000 atm hydrostatic prepressurization¹².

In the lower saturation range used here, where the number of bubbles is relatively small, motes may conceivably play a role. Therefore, it is difficult to establish with precision the minimum saturation which is necessary for spontaneous cavitation. It is difficult also to know accurately the supersaturation (that is, saturation pressure minus decompression pressure) which exists in the water at the moment of molecular nucleation, both because of the lack of detailed pressure measurements during decompression and the time delay between the moment of nucleation and the moment of first detection. However, considering all of these factors, the supersaturation for spontaneous nucleation by

argon in water is at least 150 atm, and probably between 155 and 160 atm. These values are the apparent supersaturations directly based on experimental readouts. The true gas tensions in the water may be significantly lower due to the increase in gas solubility which occurs when the pressure is released during decompression. This effect varies with the various gases¹⁴⁻¹⁸ and is not well known for argon. It is, therefore, difficult here to apply the proper correction for the hydrostatic pressure component in the effect.

The nucleation threshold value obtained here is about 30 atm higher than the supersaturation which can be maintained without any cavitation taking place either at glass interface or in the water¹¹. It is within the 100–1000 atm range suggested for spontaneous cavitation in water⁸, but below the value of 250 atm which has been implied from limited experimental observations with oxygen¹⁹.

Although cavitation in the bulk water so far has been directly examined for argon only, the cavitation properties for various gases at the glass–water interface were found to be dependent on the solubility of the gas, and hence its concentration rather than its tension^{11,12}. Thus, the onset of heavy interface cavitation occurred at identical supersaturations (that is, 145–150 atm) for argon and oxygen, two gases with very similar solubilities. For the less soluble nitrogen, 180–190 atm supersaturation was required for the same degree of cavitation. For helium, which has the lowest solubility in water, the value was in excess of 320 atm. Preliminary results on methane and nitrogen obtained by the present technique indicate that the same type of solubility relationship applies directly to the nucleation thresholds in bulk water.

Even a simplified analysis of the present results reveals some highly significant features of the nucleation process. At the critical saturation for spontaneous nucleation, the gas pressure of the nuclei, which would effectively equal the gas tension, must exceed the inward, collapsing force of the surface tension. The critical bubble radius (R) may be expressed (in cm) as: $R = 2\gamma/\Delta P$, where γ is the surface tension (dyn cm⁻¹), and ΔP is the difference (in μ bar) between the hydrostatic and the internal gas pressure. Using this conventionally accepted Laplace formula and a 'normal' surface tension (72.8 dyn cm⁻¹ at 20 °C), the minimum diameter of a stable or expanding nucleus would be 1.8×10^{-6} cm at 160 atm. Such a nucleus would contain at least 10^4 molecules, conservatively estimated by dividing the sphere volume (3.1×10^{-18} cm³) by the cube of intermolecular distance in the compressed gas phase (2.5×10^{-22} cm³). Considering the very dilute concentration of the dissolved gas at this pressure, only about 2×10^{-4} mol dm⁻³, the nearly instantaneous formation of such large gas bodies would be highly improbable, if not impossible. Aggregations brought about by fusions of unstable nuclei conceivably could reduce to some degree the dimensions for critical bubble size, but alone they could not significantly alleviate the basic problem. Also, the spontaneous formation of 'vapour' nuclei of the magnitude indicated above by the self-dispersion of water molecules must be dismissed even though void cavities involving only a few molecules have been postulated to exist in water (refs 9, 20–22).

How then does nucleation occur at the observed supersaturation values? Although it is as yet not possible to propose a quantitative model, or even to describe all of the factors involved, it appears that the surface tension at the gas–water boundary of the nucleus must be at least an order of magnitude lower than that obtained by extrapolation from macroscopic dimensions. The interaction between the compressed gas in the nucleus and the enveloping water would contribute to such a lowering²³, as would the phase boundary curvature effect²⁴, but these probably could not account for all of the effect. A less important factor which conceivably could aid the expansion of some small nuclei (and the collapse of others) would be the 'pres-

sure' fluctuations in their gas phase which consists of relatively few gas molecules. However, due to the ready diffusion of gas, no long-term imbalance could exist between the gas pressure and the gas tension in the water.

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X-ray analysis of Co–C bond cleavage in the crystalline state

DURING serial structure analysis of cobaloxime derivatives¹, aimed at interpreting the catalytic capability of the asymmetric hydrogenation²⁻⁵, we have found that the crystal of *R*- α -cyanoethyl (*S*- α -methylbenzylamine)-cobaloxime changes its unit cell dimensions by X-ray exposure without degradation of a single crystal form. The rate of the change was so slow that it was possible to collect the intensity data for several intermediate stages. We have proved, by calculating electron density in each stage, that the change reflects the racemisation of the cyanoethyl group.

Figure 1 shows the changes of unit cell dimensions a , b , c and β with exposure time to the conventional MoK α radiation. The cell dimensions at 2 h after the initiation of the irradiation are $a=8.665(2)$, $b=13.485(3)$, $c=9.584(3)$ Å, $\beta=96.95(3)^\circ$ and $V=1111.6(5)$ Å³. The a and c axes contract slightly whereas the values of b and β increase to a considerable extent. The rate of change is rapid at very initial stage, but becomes moderately slow. They converge gradually to 8.637(2), 13.833(3), 9.539(2) Å, 99.07(3)° and 1125.5(4) Å³, respectively. After 22 d the changes are within the experimental errors. The space group, P2₁, remains unaltered. The reproducibility was confirmed by runs using three different crystals. The same results were obtained for the crystal of *S*- α -cyanoethyl (*R*- α -methylbenzylamine)-cobaloxime, which is the enantiomer of the present complex.

During these experiments, the appearance of the crystals gave no evidence of decomposition or degradation and the width of each reflection did not change appreciably. The peak and integrated intensities, however, varied to a considerable extent; they fell to an average 50% and 85% of initial values respectively in intermediate stages but fully recovered at the final stage. If the irradiation was interrupted, the change of the cell dimensions became undetectable. By resuming the irradiation, it continued as before.

The three-dimensional intensity data were collected at the stages denoted as A, B, C, D, E, F in Fig. 1. Before A, intensity data collection was unsuccessful. Figure 2 shows the crystal structure, projected along the *c* axis, deduced from the data at stage A. Fairly loose contacts are observed around the methyl of cyanoethyl group.

The electron density for stage B indicated that the occupancy of the methyl of the cyanoethyl group decreased significantly and a new peak appeared. Figure 3*a* shows a part of the composite difference Fourier map. The new peak is about twice as high as that of the hydrogen atom and is comparable to the depth of the trough at the position of the methyl group. Between stages B to F, the peak grows higher, and the trough becomes deeper. Figure 3*b* shows part of the composite electron density map at the final stage

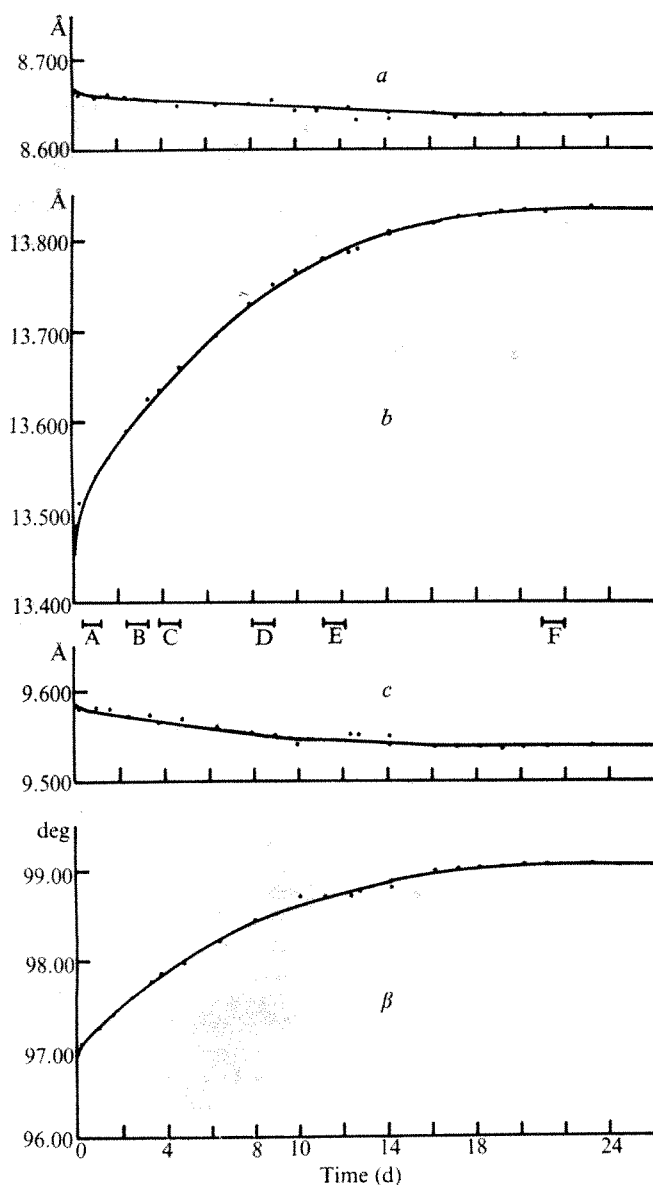


Fig. 1 The change of the unit cell dimensions. A dark red crystal, $0.2 \times 0.2 \times 0.4$ mm, was mounted on a Rigaku automated four-circle diffractometer using MoK α radiation (40 kV, 20 mA; $\lambda = 0.71069$ Å) monochromatised by graphite. Cell dimensions were determined by least-squares analysis with 15 high-angle ($2\theta > 20^\circ$) reflections. Each value is shown with a point. The experiments were done at intervals of 2 h in the initial stage and done every day in the intermediate stages. By exposure over 22 d, each value fluctuated within its experimental error. E.s.d.s are 0.002–0.004 Å and 0.03–0.05°. At the initial stage the density calculated for $Z = 2$ is 1.388 g cm^{-3} , whereas the density observed by flotation is 1.388 g cm^{-3} . Intensity data were collected at six different stages denoted as A, B, C, D, E, F.

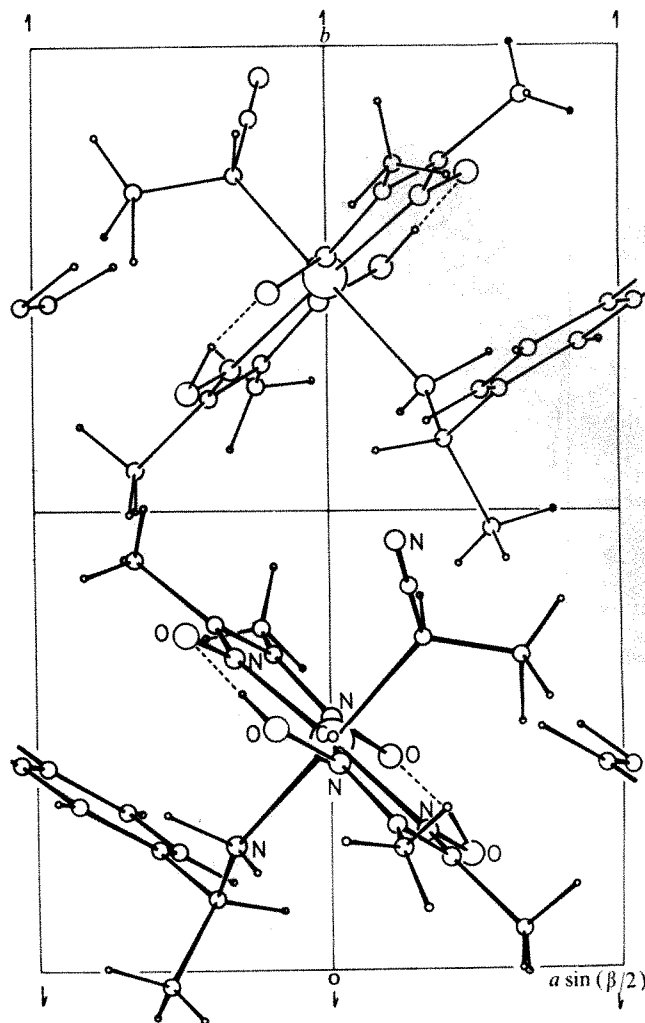


Fig. 2 The crystal structure at the stage A, projected along the *c* axis. Intensity data for independent reflections were collected with 2θ - ω scan, a scan rate of $8^\circ (2\theta) \text{ min}^{-1}$ and background counts of 5 s on both sides of the peak. Because 30 h were required for data collection, the orientation matrix of the crystal was redetermined for several times. A total of 2,069 reflections were measured within the limit $2\theta = 50^\circ$, of which 129 reflections for $|F_o| < 3\sigma(|F_o|)$ were considered unobserved. The structure was solved by the heavy atom method and refined by the block-diagonal least-squares. Final *R* was 0.055 for 1,940 reflections. The structures for other stages were determined as described above.

F. The height of the new peak is approximately the same as that of the methyl group.

These observations indicate that some portion of the methyl groups is transferred to the position of the new peak so that disordering occurs with the methyl group. The position of the new peak corresponds approximately to that of the methyl group if the absolute configuration of the cyanoethyl group is converted from *R* to *S* with the cyano group fixed, although distorted from the ideal geometry because of the repulsion with the equatorial ligands. The least-squares calculations showed that the occupancy factors of *R* and *S* methyl groups are 0.75 and 0.25 respectively at the stage B and became equal at the final stage F.

In addition to the disordering of the methyl group, the molecule undergoes gradual translation. At the final stage it moves apart from the original position by 0.04 Å in the *a* and *c* directions. No significant changes were observed in bond distances and angles between the structures at the stages A and F.

Although the mechanism of the present transition in the crystalline state is not yet established, it is clear that any of

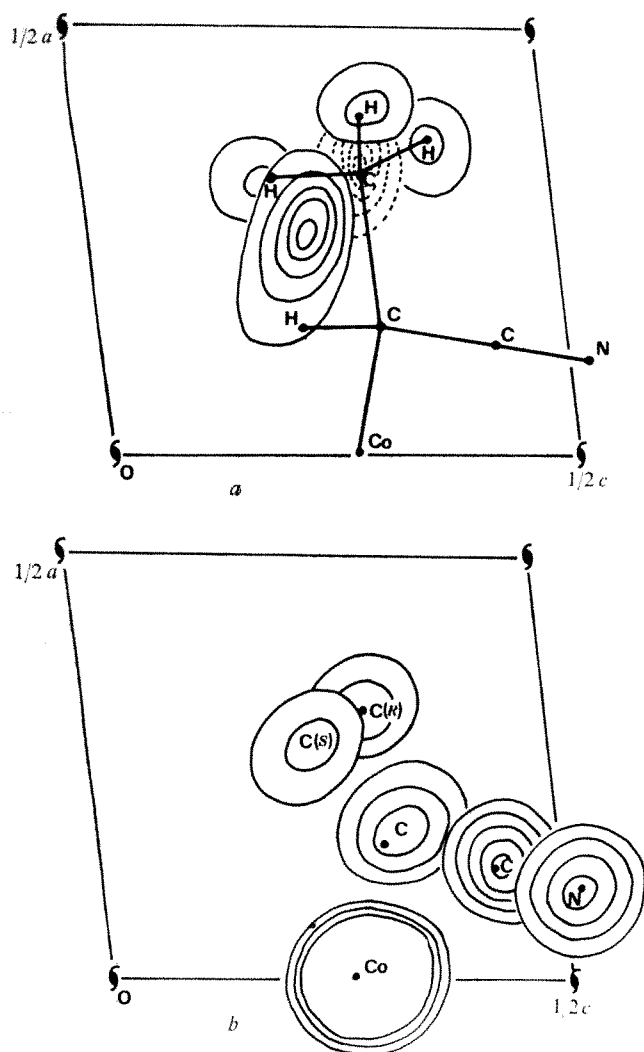


Fig. 3 *a*, Composite difference Fourier map along the *b* axis. Contour interval $0.1 \text{ e } \text{\AA}^{-3}$; negative contours broken. The analogous map was obtained at each of other stages, though the positive and negative peaks of the new peak and the methyl group respectively are enhanced and the hydrogen peaks are more obscure than those in the figure. In F_c calculation, the carbon atom of the methyl group is taken to have the occupancy factor of 1.0 and the hydrogen atoms of the methyl group are not included. The structures at the stage A are shown with the solid lines for comparison. *b*, The composite electron density map. Contour interval $1 \text{ e } \text{\AA}^{-3}$; inner contours of the cobalt atom are omitted for clarity. The black circles show the position of the corresponding atoms at the stage A. The asymmetric carbon and nitrogen atoms seem, from the spread and height of the peaks, to be slightly disordered.

four bonds, Co–C, NC–C, H_3C –C and H–C, would be cleaved in the transition; otherwise the asymmetric carbon of *R* configuration could not be converted to *S*. Schrauzer *et al.*⁶ and Giannotti *et al.*^{7–10} showed from the e.s.r. spectra that the irradiation of visible light ($\lambda > 420 \text{ nm}$) results in the homolytic cleavage of Co–C bond in the alkylamine-cobaloxime. It seems quite plausible that for the present complex the same reaction should occur in the secondary process initiated by X rays. Energy released by the bond cleavage might be given to the crystal lattice and contribute to causing topical melting. The cyanoethyl radical would be permitted to rotate to face the opposite side of the plane to the cobalt. This would result in the inversion of the configuration of the cyanoethyl group when the radical makes a bond to cobalt again. As it is quite probable that the racemic structure is more stable thermodynamically,

the growth of the racemic structure would continue without destroying a single crystal form—the racemers having almost identical cell dimensions—so that a whole crystal is converted into the racemic structure.

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A physicochemical mechanism for magnetic field detection by migratory birds and homing pigeons

MIGRATORY birds and homing pigeons can apparently obtain directional information from the Earth's magnetic field. The effect is difficult to detect, and discussion of the possible process of magnetic field detection by birds seems so far to have foundered on the simple fact that the orientational effect of the Earth's magnetic field on a single electron spin associated with a molecule of animal tissue would be of the order 10^{-8} eV —almost certainly too small to be detectable biologically. Here I direct attention to a process which would overcome this basic problem, and which also seems to provide an explanation of all the main features of published data. It is a mechanism in principle only, however, and is discussed here in no more detail than is necessary to clarify the basic ideas and to provide a basis for further investigation.

There are three requirements that any proposed physicochemical mechanism for field detection must satisfy. First the mechanism must overcome the minuteness of the orientational energy produced by a magnetic field as small as that of the Earth's. Second, the mechanism must be basically unlikely, to account for the weakness of the observed response. Third, if the response is to display the necessary anisotropy (so that the bird prefers one direction to another), detection at the molecular level must take place in an ordered array of molecules, each with an anisotropic response to an applied magnetic field.

A mechanism which satisfies these requirements represents an application of optical pumping which depends on the presence and detection of radiation in the visible region of the spectrum. I propose therefore that magnetic field detection in birds takes place in the eye, in the molecules of the retina (a comparatively well-ordered structure spatially) as an adjunct to the normal processes of vision. Specifically, I propose an optical/r.f. (radio frequency) double resonance process involving the lowest excited molecular triplet state of (say) the rhodopsin molecule. The normal visual process begins with the absorption of a photon in the visible region. For various molecules such

an absorption is a singlet-singlet transition between the molecular ground state and a vibrationally broadened singlet excited state³. Neither of these states, being singlet ($S = 0$), have a magnetic moment, but the triplet states do, having total spin $S = 1$. For such states the anisotropy in the spin-spin dipolar interaction lifts the spin degeneracy in a manner most conveniently characterised by the effective spin-Hamiltonian parameters D and E (refs 4, 5). In conditions of continuous irradiation these triplet levels are dynamically populated through inter-system crossing from the singlet levels through spin-orbit interaction⁶, and experiment has shown that the relative populations of the three triplet states may be appreciably different from unity, depending on the details of symmetry and the relative probability of radiative transitions to the ground state^{6,7}. The relevance of this is that (1) the energy variation of triplet states with field is anisotropic, depending on field magnitude as well as the field direction relative to the principal axes of D and E ; (2) radiative transitions from these states back to the ground state are forbidden transitions and therefore weak; and (3) such transitions have definite polarisation relative to the molecular axes, and the intensity of each transition would depend on the relative populations of the triplet states. If, therefore, the next stage of the detection process were able to monitor radiative intensity at a particular polarisation, and if the relative populations of the triplet states were to vary with magnetic field direction, the detection process would be possible in principle. For this latter requirement we have to postulate first that the parameters D and E produce the necessary magnetic anisotropy, and second, that at least one of the anisotropic $S = 1$ splittings comes into resonance with some internal relaxation process having a frequency around 3 MHz (to satisfy $h\nu_{rr} = g\beta B$, with $B \approx 10^{-4}$ T). It would be premature to try to specify the possibilities for this at present; optical pumping is a double resonance process and therefore depends not only on the existence of such a resonance but also on various other relaxation rates happening to be within a suitable range. Typical values for D and E for various molecules already studied^{4,6} are two to three orders of magnitude too large, though the requirement here is merely that E should be negligible compared with splittings that would be produced by the Earth's field, while D could take any value. Radiationless decay rates vary widely, but tend to be too fast rather than too slow. The model does therefore face some considerable difficulties in detail, though direct evidence for rhodopsin, or whatever molecule is active in the living creature, is lacking at present. One might therefore have been reluctant to postulate that birds were able to detect the Earth's field, but here one is faced with the fact that they do. I now turn to a review of the experimental data in the light of the above model.

The main findings seem to be: (1) response to field is evident over a wide range of illumination—daytime overcast¹¹ to starlit sky¹⁷—but not, so far as I am aware, in total darkness (see (7) below). This is consistent with a detection process based on the normal visual process. (2) The response is much weaker than other directional clues (though stronger, apparently, than stellar constellation information¹⁷) and is difficult to detect experimentally. This is consistent with the electric-dipole forbidden nature of triplet state radiative decay, and the probability of competing radiationless decay processes. (3) The response to magnetic field is axial rather than polar. That is, birds show sensitivity to the angle of dip rather than the N-S direction of the field⁸⁻¹⁰. This is consistent with a resonance process (though there are slight difficulties concerned with the detection of a particular polarisation in the next stage of the process) and is perhaps the best evidence there is for the proposed model, particularly when taken together with the fact that (4) the response to magnetic field reaches a peak for fields comparable with the Earth's, falling off at both lower and higher fields⁹. (5) The response to field disappears temporarily if the field is altered a little ($\sim 20\%$), but returns if the birds remain in the altered field for a day or two⁹. This is consistent with the detection process taking place in a directionally ordered

structure (such as the retina) and where the molecular response to field is axial; that is only the field component parallel to the molecular axis matters, as in the case $D \gg E$. In this case for a particular eye orientation the resonance condition would be satisfied on particular areas of the retina. Altering the field would shift the resonance condition to some other region of the retina and confuse the bird initially. A day later, however, it could have learnt to respond to the altered cue. (6) Birds and a large number of other creatures are able to detect polarisation of light¹². This need not necessarily have its origin in triplet state radiative decay, but it is a requirement of the proposed model. (7) Attempts to operantly condition pigeons to magnetic field have so far failed to produce unambiguous results^{13,14}. A possible reason for this may be found (thanks to Professor Keeton for providing further details of experiments from ref. 14) in the lighting level during the experiments. Birds go to sleep in total darkness⁹, while normal laboratory lighting is too bright for operant conditioning, so the light level is therefore used to set the bird's level of awareness to a hopefully suitable level for the experiment. If, however, field detection does take place in the eye, there is some possibility of 'tuning out' precisely the effect one is seeking, by using the light level in this manner. (8) There is somewhat sketchy evidence that birds lose their orientation in the vicinity of radar installations¹⁵. This is consistent with resonances in the GHz region due to the D splitting of the triplet state, which would tend to disturb the equilibrium due to the internal r.f. resonance as postulated.

Thus, in conclusion, optical pumping overcomes the basic problem faced by any proposed model for field detection in biological systems, and also the excited triplet state provides for the anisotropy which is essential if such detection is to be useful directionally. Though the requirements placed on the parameters D and E and all the appropriate relaxation processes are rather stringent, the straightforward explanation which this model provides for the existing data does provide some grounds for optimism. The questions it raises are obvious—is vision essential to magnetic field detection? Can the physico-chemical requirements be satisfied? Behaviourally, it might be interesting to conduct the directed-perching type of experiment⁹ in monochromatic light at long wavelengths, in the hope of modifying the excitation transfer probability to the lowest excited triplet state. Finally, nothing in the proposed mechanism is specific to birds, rather than other creatures which have likewise shown sensitivity to the Earth's magnetic field¹⁶.

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Sources of sulphur in rain collected below a wheat canopy

VEGETATION plays an important role in the cycle of sulphur between the atmosphere and the soil¹⁻⁴. We have measured the quantity of sulphur in rain collected below a maturing wheat canopy. This sulphur has three sources: first, the atmosphere, from which falling rain gains SO₂ and sulphate; second, leaf surfaces, from which rain washes sulphur which was previously deposited by turbulent transfer ('dry deposition'), and third, leaf tissue, from which rain leaches sulphur⁵. We have now deduced from field and laboratory measurements that leaching supplied nearly 90% of the sulphur gained by rain as it fell through the wheat canopy. Only a small fraction of sulphur which had been dry-deposited on the surface of leaves could be washed off.

The measurements were made in June, July and August 1975 in a 6.9-ha field of winter wheat (variety Maris Huntsman) growing at Sutton Bonington (52.8°N, 1.25°W), a rural area within an industrial region. Rainfall above the canopy was collected at 12 sites in the field in polyethylene funnels, diameter 14 cm, which drained into glass bottles. At each site, throughfall below the canopy was collected in polyvinyl chloride guttering, 2 m × 7 cm, laid along the rows and draining into glass bottles. These linear gauges were calibrated over short grass by comparison with a standard rain gauge. The funnels and gutters were wiped with tissues soaked in distilled water at least every 4 d and whenever possible immediately before rain fell. This practice may lead to underestimation of the quantity of rain received in light showers but it minimises analytical errors from contamination⁶. A linear regression of throughfall, T (mm), against rainfall, R (mm), for 11 events yielded $T = 0.50R - 0.036$ ($r = 0.99$). Experiments with artificial rain indicated that the canopy storage capacity immediately after rain was 0.5 mm. We were unable to measure stemflow, S , so we assumed that it accounted for the residual, that is $R - 0.5 = S + T$. The large stemflow component is consistent with the upright structure of the crop and the compositions of stemflow and throughfall were assumed to be identical because both passed over similar leaf surfaces.

The wheat crop was sampled at weekly intervals by collecting tops from four randomly selected rows, 2 m long, and roots from four soil cores to 2-m depth. The four top samples were digested individually; roots were washed free of soil and digested in bulk⁷. The sulphur contents of digested plant samples and of rainfall were determined by precipitation of barium sulphate. Washing followed by redissolution of the precipitate in EDTA facilitated the measurement of barium by atomic absorption spectrophotometry.

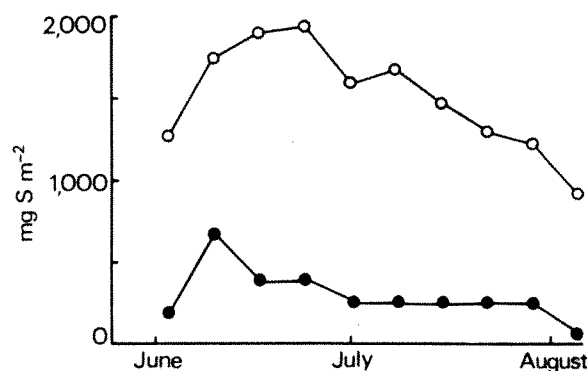


Fig. 1 Sulphur content of winter wheat in 1975 expressed as sulphur per unit ground area ●. Roots; ○. roots plus tops. Anthesis was on 15 June and harvest on 5 August.

Table 1 shows that after rain additional sulphur was always found in water collected below the canopy. The excess consists of dry deposition washed from leaf surfaces and leachate from leaf tissue. The contributions from each source during the whole period can be assessed as follows.

Fowler^{2,8} made a detailed study of dry deposition of SO₂ on wheat growing in the same field in 1974 and deduced typical values of the deposition velocity, v_d , to wet and dry leaf surfaces⁸. The mass, M , of sulphur dioxide deposited in time, t , on leaves covering unit ground area is $M = v_d \chi t$ where χ is the SO₂ concentration above the surface. While our measurements were being made, the mean value of χ at a nearby site was $32 \mu\text{g m}^{-3}$. Meteorological observations indicated that leaves were wet (mainly with dew) for about 15% of the 66-d period. Using Fowler's values $v_d = 1.5 \text{ mm s}^{-1}$ (dry) and 10 mm s^{-1} (wet) we estimate that M corresponded to about 250 mg S m^{-2} during the whole period, that is deposition on the cuticle was similar in magnitude to the 236 mg m^{-2} additional sulphur found below the canopy (Table 1).

To estimate the fraction of deposited sulphur which could later be washed off the cuticle, we exposed leaves of senescent wheat plants growing in pots to radioactive ³⁵SO₂, removed the leaves at intervals ranging from 10 min to 8 d, and washed leaf surfaces thoroughly with distilled water. Stomata in senescent leaves present a large resistance to gas exchange⁹ so we assume that most of the radioactive sulphur was on the cuticle. The fraction of total activity which could be washed off had a mean of 0.13 and ranged from 0.04 to 0.19. If the same mean figure is valid in the field, dry deposition washed from leaves supplied only about 30 mg S m^{-2} of the additional 236 mg S m^{-2} recorded below the canopy (Table 1). Garland and Branson found a similar fractional washoff from pine shoots exposed to ³⁵SO₂ (ref. 10).

Table 1 Sulphur contents of rainfall and throughfall below a wheat canopy

Period of rainfall	Rainfall (mm)	S content of rain ($\mu\text{g ml}^{-1}$)	S input to canopy in rain (mg m^{-2} ground area)	S content of throughfall ($\mu\text{g ml}^{-1}$)	S output from canopy by throughfall and stemflow (mg m^{-2} ground area)
2-3 June	4.3	$3.1 \pm 0.2^*$	13.3	3.6 ± 0.2	13.3
15-16	10.7	3.3 ± 0.3	35.3	5.0 ± 0.4	51.0
8-10 July	1.6	13.9 ± 1.1	22.4	34.3 ± 2.6	37.7
10-13	5.6	3.4 ± 0.2	19.0	24.8 ± 2.6	126.5
13-15	9.1	2.0 ± 0.1	18.2	6.8 ± 0.7	58.5
15-18	16.9	2.2 ± 0.1	37.2	3.5 ± 0.2	57.4
18-21	11.3	3.3 ± 0.1	37.3	6.0 ± 0.4	64.8
21-23	2.9	3.0 ± 0.2	8.7	5.3 ± 0.5	12.7
4-5 Aug	1.3	11.1 ± 0.4	14.4	25.3 ± 2.2	20.2
			Total 205.8		Total 442.1
					Additional sulphur 236 mg m^{-2} ground area

*s.e.m.

Figure 1 shows the results of the sulphur analysis of whole plants and roots expressed as sulphur per unit ground area. Clearly sulphur losses from the plants after anthesis (15 June) greatly exceeded sulphur additions measured in throughfall. The sulphur content of the tops decreased by about 40% after this time, but Table 1 shows that leaching from leaves accounted for only about one third of the sulphur lost. Because sulphur did not accumulate in roots we conclude that it was removed by other mechanisms as well as by leaching in rain. Exudation of sulphur from roots of plants growing in solution and exposed to SO_2 has been reported¹¹⁻¹³. Knowles and Watkin¹⁴ observed large losses of potassium (approximately 50% of maximum content) from wheat plants after anthesis and they postulated an efflux through the roots to the soil. A similar efflux of sulphur seems the most likely explanation of our results. Emission of gaseous sulphur compounds from leaves (reported for tomato plants¹⁵ and pine needles¹⁶) is another possibility.

Our measurements and calculations show that leaching of plant sulphur by rain was the main source of additional sulphur in throughfall, and that most sulphur dry-deposited on leaf surfaces remained fixed there. Current studies in Scandinavia aim to deduce deposition velocities for SO_2 to forests by ascribing part of the excess sulphur in throughfall to deposited sulphur washed from leaf surfaces³. Our results show that this technique would be highly inaccurate for a maturing wheat crop, and it is clearly desirable to make further studies with vegetation of other ages and species to establish the pathways in the sulphur cycle more clearly.

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Seed germination in response to diurnal fluctuations of temperature

DIURNAL fluctuations in temperature may initiate or accelerate germination in certain flowering plants¹⁻⁶, and the effectiveness of the stimulus varies according to the amplitude of fluctuation⁷ and the presence or absence of light⁸. Attempts^{3,6} to assess the adaptive significance of the phenomenon, however, have been limited by the scarcity of data for species of contrasted ecology. We report here an investigation of germination responses to fluctuating temperatures, conducted on seeds of herbaceous species collected from native populations near Sheffield. The results suggest that requirements for diurnal fluctuations in

temperature are characteristic of the germination of species from particular types of habitat and provide mechanisms which cause seeds to germinate at times and in places favourable for seedling establishment.

Species were screened for the capacity to respond to fluctuating temperatures in the light in two stages. First, germination was compared in two incubators. One consisted of a growth room with a diurnal fluctuation of 5 °C (20 °C day, 15 °C night) and the other was a thermogradient bar⁹ providing 'constant' temperatures (maximum diurnal fluctuation 1 °C) for 30 batches of seeds distributed at regular intervals across the range 5-40 °C. This procedure revealed a large number of species which germinated to a high percentage in fluctuating temperatures but which remained partially or totally dormant in constant conditions. Such species included several marsh plants, in some of which germination on the thermogradient bar was restricted to exceptionally high (>25 °C) temperatures.

In the second stage of screening, germination of each of these species was examined in a new incubator⁷ which provides diurnal temperature fluctuations over a selected range of amplitudes. The results described here refer to experiments in which batches, each of 30 seeds, experienced diurnal fluctuations over the amplitude range 0-12 °C. Details of the methods are given in the legend of Table 1. Each fluctuation was applied in the form of a depression

Table 1 Species in which germination in the light is promoted by fluctuating temperatures

Species	Fluctuating temperature requirement (°C)
<i>Agropyron repens</i> *	4.0
<i>Atriplex hastata</i>	6.5
<i>Bidens tripartita</i> *	4.0
<i>Cardamine pratensis</i>	7.0
<i>Carex otrubae</i>	1.0
<i>Chenopodium rubrum</i>	7.0
<i>Filipendula ulmaria</i>	1.5
<i>Gnaphalium uliginosum</i> *	5.0
<i>Juncus effusus</i>	1.5
<i>Lycopus europaeus</i> *	8.0
<i>Mentha arvensis</i> †	4.5
<i>Phalaris arundinacea</i>	1.0
<i>Polygonum persicaria</i>	8.0
<i>Ranunculus repens</i> ‡	1.0
<i>Rorippa islandica</i> *	9.0
<i>Rumex obtusifolius</i>	2.0
<i>Rumex sanguineus</i> *	2.5
<i>Sieglingia decumbens</i> *	1.0
<i>Sonchus arvensis</i>	7.5
<i>Tripleurospermum maritimum</i>	1.5
<i>Typha latifolia</i>	1.0
<i>Urtica dioica</i>	5.5

The figures indicate the amplitude of fluctuation required to attain 50% of the maximum germination recorded in the experimental conditions. Germinated seeds were counted and removed daily. Diurnal fluctuations were maintained throughout each experiment, which was terminated when germination ceased. Light intensity during the light period was 1,846 $\mu\text{W cm}^{-2}$. Before the test all seed was stored dry at 5 °C except for the following.

*20 °C dry storage.

†2 °C wet storage.

‡20 °C wet storage.

Further studies have indicated that requirements for fluctuating temperatures in certain of these species may decline gradually during extended laboratory storage.

below a base temperature of 22 °C extending for 6 in every 24 h and coinciding with a dark period. Such downward fluctuations were preferred so that no regime should impinge on the high temperatures known to initiate germination in certain of the species. Thus any responses could be attributed to the fluctuation itself rather than to experience of particular absolute temperatures.

In Table 2, the 292 species screened are classified arbitrarily into five ecologically defined groups, for each of which the proportion of species responding to fluctuating

temperatures in the light has been calculated. Some of the species stimulated by fluctuating temperatures are identified in Table 1, which also includes the amplitude required to achieve 50% of the maximum germination recorded in the experimental conditions. A high incidence of responses to fluctuating temperatures is apparent in species of marshes, bogs, streambanks and other wetland habitats. The significance of this correlation is strengthened by the fact that several ruderal species in which germination was found to depend on fluctuating temperatures, such as *Chenopodium rubrum*, *Polygonum persicaria* and *Polygonum lapathifolium*, are particularly common in areas of arable fields subject to seasonal waterlogging, and frequently colonise the mud adjacent to lakes, ponds, ditches and reservoirs¹⁰.

Further investigations with modified incubators were conducted to examine the response of seeds to diurnal temperature fluctuations in light and in darkness. Specimen results are shown in Fig. 1. In some marsh plants and ruderals darkness increased considerably the amplitude of fluctuation required to initiate germination. For many species which in light germinated rapidly and to a high percentage in constant temperatures, a large proportion of the seeds required large (>4 °C) fluctuations to germinate in darkness. This category included species from grassland, heathland and a wide range of vegetation types.

Table 2 Comparison of frequency of occurrence of requirements for fluctuating temperature for germination in the light

Habitat	No. of species examined	% Stimulated by fluctuating temperatures
Wetland	68	43
Disturbed ground	61	18
Grassland	100	2
Woodland	20	5
Remainder	43	0

Further experiments are necessary to estimate how far these results from local seed collections are representative of each species. Already, however, responses in two ecologically distinct groups are evident. The first (group 1) consists of species (mainly marsh plants) which require fluctuating temperatures for germination in the light. The second (group 2), including plants from a wide range of habitats, consists of species in which the response to fluctuating temperatures occurs only in conditions of continuous darkness or is greater in darkness than in the light. We suggest that in the two groups responses to temperature fluctuations are initiated by different environmental cues.

We consider first group 1. In temperate regions, seedling establishment in many marshland species is restricted to late spring when the water table recedes, causing bare soil to be exposed and its surface horizons to become aerobic. We suggest that fluctuating temperature requirements delay germination until such time in the spring that the declining water content of the soil causes the seed to be no longer insulated against the increasing daily radiation load.

The necessity of bare soil for seedling establishment is not confined to ruderal species. Ecologists¹¹⁻¹⁴ have noted that seedlings of many group 2 species establish successfully only in gaps in the canopy of leaves and litter. From studies of the seed content of soils¹⁵⁻²⁰ and our own measurements it is apparent that buried seeds of many herbs persist in large numbers beneath closed canopies. Such species include all those which in Fig. 1 display a requirement for fluctuating temperatures in darkness. We suggest therefore that germination is initiated in gaps in vegetation by two processes: (1) unearthing of buried seeds and triggering of germination by exposure to light and (2) germination of buried seeds *in situ*, caused

by large diurnal temperature fluctuations resulting from removal of the insulating effect of foliage and litter. The results of a temperature monitoring experiment in a grass sward (Fig. 2) confirmed that at a soil depth of 1 cm, beneath a closed canopy, diurnal fluctuations in temperature exceeded 4 °C only in exceptionally hot dry conditions, whereas, in neighbouring gaps, amplitudes greater than 10 °C were frequent.

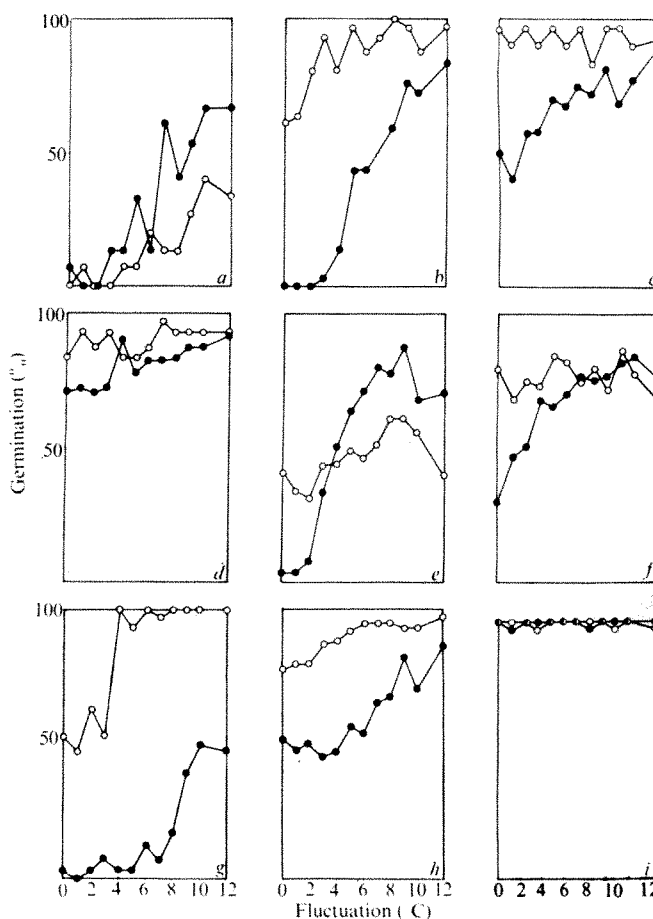


Fig. 1 Germination responses to various amplitudes of diurnal fluctuations of temperature in light (○) and in continuous darkness (●) in nine herbaceous plants. Details of the experimental procedure are provided in the legend of Table 1. The erratic course of the 'curves' for certain species, for example, *A. repens*, probably reflects the heterogeneity of responses within the seed populations studied. In any sample of such seed there exists a mixture of phenotypes differing in response to temperature fluctuations. Such variability could be eliminated only by the use of very large numbers of seeds in the experiments. The results for *A. repens* and *P. pratensis* suggest that even when exposed to large fluctuations of temperature, a proportion of the seeds was inhibited by the light treatment. This suggests a mechanism whereby germination of seeds of these species may be delayed until after burial in the soil. a, *A. repens*; b, *D. caespitosa*; c, *H. lanatus*; d, *P. annua*; e, *P. pratensis*; f, *P. trivialis*; g, *R. obtusifolius*; h, *S. media*; i, *L. perenne*.

Control of arable weeds is complicated by the erratic germination patterns exhibited by certain species. It seems likely that one reason for the low predictability of germination in species such as dock (*Rumex obtusifolius*), couch grass (*Agropyron repens*) and chickweed (*Stellaria media*) is the heterogeneity of their seeds with respect to the amplitude of fluctuation required to induce germination in darkness (Fig. 1). In field conditions such variability is compounded by the fact that seeds are distributed throughout the soil and their exposure to temperature fluctuations will be modified by variations in incident radiation, vegetation canopy and soil moisture content.

Responses of buried seeds to fluctuating temperatures

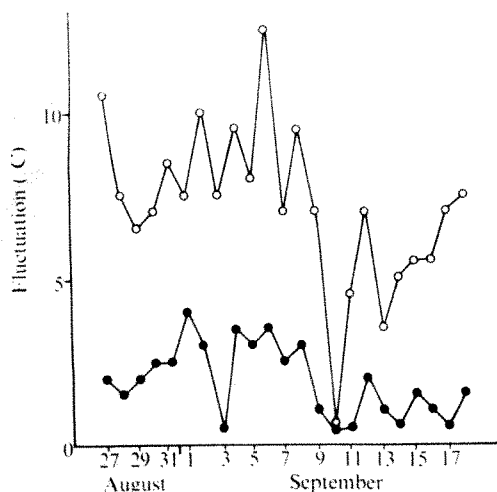


Fig. 2 Comparison of the amplitudes of diurnal fluctuations of soil temperature beneath an established sward (●) and in an artificially created gap of 20-cm diameter (○). Measurements were made at a depth of 1 cm. Recording was carried out during the period 27 August to 18 September 1976. Each point refers to the difference between the minimum and maximum temperatures on the day concerned.

are also relevant to the 'deterioration' of pastures, characterised by a decline of the sown species (usually *Lolium perenne*) and an incursion by native grasses. Figure 1 shows that in native sward-invading species such as *Poa annua*, *P. trivialis*, *P. pratensis*, *Holcus lanatus* and *Deschampsia caespitosa*, a proportion of the seeds require large temperature fluctuations for germination in darkness. It is not surprising therefore that these species occur in the reservoir of buried seeds in pastures¹⁶ and exploit gaps in the canopy arising from damage to the vegetation. The decline of *L. perenne* may be related in part to the fact that it is insensitive to diurnal temperature fluctuations and is not incorporated into the buried seed flora.

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Energy requirement for symbiotic nitrogen fixation

COMPARING the energy required by legumes for symbiotic nitrogen fixation with that of assimilation of nitrate, Gibson¹ concluded the costs to be about the same. About 15% of net photosynthetic production by the plant may be used in meeting its nitrogen requirements. If energy for the fixation of nitrogen symbiotically and that for the assimilation of NH_4^+ or NO_3^- from the soil solution are both provided by the chemical products of photosynthesis, then the CO_2 respired in supplying that energy must contribute to the total efflux of CO_2 from the plant in the dark. McCree² and Thornley³ have discussed methods of partitioning the dark CO_2 efflux into a growth (synthesis) and a maintenance component. Both nitrogen fixation and nitrogen assimilation can be expected to contribute to the CO_2 flux associated with synthesis. If Gibson is correct, nodulated plants using only symbiotically fixed nitrogen should have the same growth coefficient as non-nodulated plants supplied with exogenous mineral nitrogen when grown in the same conditions. I have examined this hypothesis using a modification of the method used by McCree with *Trifolium subterraneum* L. cultivar Woonenellup as test material.

Plants were grown at a density of 2,000 m^{-2} in square, 3-l plastic pots. The rooting medium was a fritted clay of excellent water holding capacity, low bulk density (450 kg m^{-3}) and with no measurable capacity to absorb or evolve CO_2 . Plants in one group of pots (A) were inoculated with *Rhizobium* and watered daily with a nutrient solution free of mineral nitrogen. The other group (B) was not inoculated and was watered with the same nutrient solution but with 7.5 mM NO_3^- partially replacing SO_4^{2-} . Pots were housed in a growth cabinet set at $20 \pm 0.5^\circ\text{C}$ and plants were exposed to a photosynthetic photon flux density of 500 $\mu\text{E s}^{-1} \text{m}^{-2}$ for 12 h each day. Plants subjected to the two treatments grew equally vigorously. After a full canopy had developed, the CO_2 exchange rates were measured in an assimilation chamber (open system) at 20°C and the same light flux density as the growth cabinet. CO_2 flux into and out of the whole pot was measured continuously and the total daytime influx (D) and night efflux (N) were obtained by integration. This was done for three successive 24 h periods, each pot always reaching a steady state of near constant D and N over the second and third periods. At the beginning of the fourth day the light was turned off, the plants remaining in darkness for a further 36 h. The rate of CO_2 efflux declined rapidly over the first 4 h of extended night, reaching a new, steady, low rate at the beginning of the next day: a new total 12-h efflux (N_a) was calculated from this reduced value. N_a was always about half N.

The method of estimating the growth coefficient k in the equation

$$N = kD + (1+k)cW \quad (1)$$

will be described more fully elsewhere. It is assumed^{2,4} that N_a represents the maintenance flux and is equal to cW where W is the total plant dry weight in CO_2 equivalents, and c the maintenance coefficient (mg CO_2 per g CO_2 per 12 h). The growth coefficient k represents the fraction of the net CO_2 uptake in the light (D) that is associated with the synthesis of new dry matter (g CO_2 per g CO_2) over a 12-h period.

$$k = (N - N_a)/(D + N_a) \quad (2)$$

Values of k and c averaged over six replicates were:

	k	c
A (nodulated)	0.189 ± 0.017	11.9 ± 2.0
B (non-nodulated)	0.137 ± 0.010	13.2 ± 1.8

The two values of k are highly significantly different in a t test: those of c do not differ. A value of $k = 0.144$ has been obtained for non-nodulated plants grown elsewhere in the same conditions.

Assuming the dark CO_2 efflux (that is, N) to be the same during the 12-h day as during the 12-h night, these results show that plants fixing nitrogen symbiotically from the air used 37.8% (that is 2 k) of the net daily CO_2 uptake for synthesis of new material in 24 h. Those assimilating mineral nitrogen from the soil solution used 27.4% in 24 h. Nodulated plants averaged 3.9% nitrogen, the non-nodulated 4.5%, these values being each a weighted mean for the whole plant. Nodulated plants used 810 mg of CO_2 for the synthesis of 1 g of dry weight of plant material whereas for non-nodulated the figure was 510 mg. The latter is slightly higher than the value of 494 mg calculated from data of Penning de Vries⁵ for a proximate composition of 1 g of clover biomass as protein, 0.24; carbohydrate 0.61; lipid 0.07; minerals 0.08. Calculation of the conversion coefficient (Y_G)³ by the equation:

$$Y_G = (D - N) / [(D - N) + 2(N - N_a)] \quad (3)$$

shows nodulated plants to have the value of 0.64 ± 0.14 and non-nodulated plants the value 0.72 ± 0.14 , the latter being very close to the expected value of 0.74.

I conclude from use of the method of CO_2 exchange described here that the energy requirement for symbiotic nitrogen fixation by subterranean clover is substantially greater than that required for the assimilation of mineral nitrogen from the soil.

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Krameria, free fatty acids and oil-collecting bees

VOGEL'S report^{1,2} of the production of glycerides rather than nectar as rewards gathered by bees described an association involving several genera of insects and numerous plant families. We report here that members of the *Krameriaceae* produce floral lips more unusual than those described by Vogel². Our data also provide evidence that the oils of *Krameria* are collected by bees of only one genus, *Centris* (Anthophoridae), and used, mixed with pollen, as larval food.

The *Krameriaceae* is a monotypic family of about 17 species native to the New World arid and semi-arid regions from Kansas, USA to northern Argentina and Chile. The flowers of all species have five (or four) rather showy sepals (Fig. 1a, s) in a zygomorphic arrangement reminiscent of a caesalpinaceous legume flower. The true petals are dimorphic. Three (or two) of the petals are small, but petaloid, often fused at the base, and form a 'flag' above the ovary (Fig. 1a, p). The remaining two petals are highly modified into fleshy secretory glands, one on either side of the ovary (Fig. 1a, g). Two externally distinctive kinds of glands occur in two species groups within the family. One type (Fig. 1b) has bulbous swellings over the densely secreting areas of the lip and upper portion of the gland. The second type (Fig. 1c) secretes oil over the entire pebbled outer surface. Glands of both types produce the oils through a row of elongate epidermal cells (Fig. 1d). The oils collect under the cuticle, which, separates from the epidermal cells, forming lipid-filled 'blisters'.

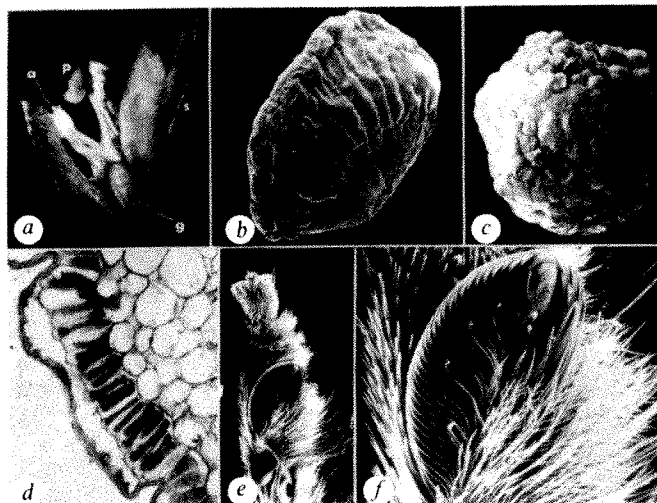


Fig. 1 A *Krameria* flower, oil-producing glands and scraping devices of oil-collecting *Centris*. a, Flower of *K. revoluta*: a, anthers; p, petals; s, sepals; g, gland ($\times 4$). b, Gland of *K. revoluta*, freeze dried, photographed under scanning electron microscopy, (SEM) ($\times 14$). c, Gland of *K. grayi*, critical point dried, photographed under SEM. Where the cuticle has been scraped away, the epidermal secretory cells are visible ($\times 14$). d, Cross section of the edge of a gland of *K. revoluta* showing the secretory epidermal cells and the separated cuticle, light microscopy ($\times 180$). e, Left front leg terminal segment of a female *Centris* (*Paracentris*) *aterrima*, an oil-collecting visitor of *K. revoluta*, under SEM ($\times 18$). f, Basitarsal scraper of the leg in (e) showing the opposing rows of setae, under SEM ($\times 55$).

None of the species in the 49 genera in six plant families³ known or suspected to produce floral oils as rewards secretes nectar. In most of these taxa lipids are secreted by trichomes, but members of *Sigmatostalix* and *Oncidium* (Orchidaceae) and all the oil-producing Malpighiaceae have epidermal secreting cells similar to those of *Krameria*.

The only detailed chemical investigations of floral oils have been carried out by Vogel and collaborators² on trichome exudates of *Calceolaria pavonii*. They identified the major component of the crude exudate as a diglyceride of acetic acid and β -acetoxypalmitic acid.

We have analysed the floral lipids of several species of *Krameria* using a combination of thin-layer and gas chromatography, infrared spectroscopy, nuclear magnetic resonance (NMR) and mass spectroscopy. The crude oil eluted from glands reached 0.91 mg per gland. The extracts seemed to be quite pure and similar in all species sampled. Spectral data and thin-layer chromatography after saponification revealed no traces of glycerol and indicated that the major components were free fatty acids. Infrared and NMR spectra showed that the fatty acids were saturated and had an acetate moiety in the β position. Analyses of the methyl esters using gas chromatography indicated chain lengths between C16 and C20. Free fatty acids are rare in plants; as far as we can determine, β -acetate-substituted free fatty acids have not been reported before.

With two exceptions⁴, all bees presumed to collect floral oils belong to seven New World genera of the Anthophoridae. Our data (Table 1) indicate that only female bees of the genus *Centris* visit *Krameria* for oil collection. The basitarsi of the fore and midlegs of almost all oil-collecting female bees have modifications which facilitate the collection of oils. The size, shape, fine structure and orientation of the oil-collecting scrapers show considerable interspecific and intraspecific variation related in part to the type of oil-producing flower visited (that is those with epidermal cell glands compared with trichomes). All female *Centris* known to visit *Krameria* have a forebasitarsal scraping device (Fig. 1e) consisting of an inner apical comb

Table 1 *Krameria-Centris* associations

<i>Krameria</i> species	Locality	<i>Centris</i> species (females only)
<i>K. cistoides</i> Hook & Arn.	Chile	<i>C. (Paracentris) chilensis</i> (Spinola)
<i>K. cuspidata</i> Presl.	Chile	<i>C. (Wagenknechtia) rhodophthalma</i> Perez
	Mexico	<i>C. (Paracentris) aterrima</i> Smith
	Mexico	<i>C. (Paracentris) atripes</i> Mocsary
	Mexico	<i>C. (Hemisiella) trigonoides</i> Lepeletier
	Honduras	<i>C. (Centris) segregata</i> Crawford
<i>K. cytisoides</i> Cav.	Mexico	<i>C. (Acritocentris) n. sp.</i> (Snelling det.)
<i>K. grayi</i> Rose & Painter	Arizona	<i>C. (Paracentris) rhodopus</i> Cockerell
	Arizona	<i>C. (Paracentris) cockerelli resoluta</i> Cockerell
<i>K. lanceolata</i> Torrey	Texas	<i>C. (Paracentris) atripes</i> Mocsary
<i>K. parvifolia</i> Benth.	Arizona	<i>C. (Paracentris) aterrima</i> Smith
	Arizona	<i>C. (Paracentris) rhodopus</i> Cockerell
	Arizona	<i>C. (Paracentris) cockerelli resoluta</i> Cockerell
<i>K. ramosissima</i> (A. Gray) S. Wats.	Texas	<i>C. (Paracentris) atripes</i> Mocsary
<i>K. revoluta</i> Berg.	Mexico	<i>C. (Paracentris) aterrima</i> Smith
	Mexico	<i>C. (Paracentris) atripes</i> Mocsary

of flattened setae with a short opposing row of giant spatulate setae (Fig. 1f). There is also a less elaborate row of flattened setae on the midbasitarsi.

Our field observations of *Centris* females show that their oil-foraging behaviour is quite stereotyped. When approaching a *Krameria* flower, the female orients herself with the supra-ovarian petals and alights, straddling the stamens and stigma. She then grasps the base of these petals with her mandibles and rapidly rubs the scrapers on the forelegs over the glands, rupturing the blisters and accumulating the oil in the hollows of the scrapers. If manipulating oil glands, a female normally spends 2–5 s per flower, but she often approaches and rejects 30 or more flowers in succession before alighting. After accumulating oil from several flowers, she transfers it, while hovering or perched on a twig, to the scopal hairs of the hind legs. The oils can be held alone by capillary action or mixed with pollen for transport to the nest.

The oils seem to be used solely as part of the larval nest provisions. Like most members of the Anthophoridae, members of the genus *Centris* are solitary bees with each female building and provisioning her own nest cells^{3,4}. Each urn-shaped larval cell is lined with a wax-like substance and half filled with a mixture of semifluid pollen and oil, on top of which the egg is laid. Our analyses of the contents of a complete nest cell of *C. trigonoides* from Pochutla, Mexico, showed no nectar (no appreciable sugar) and large quantities of lipids mixed with pollen.

Most species of *Centris* are catholic in their choice of pollen hosts, but they do not specifically collect *Krameria* pollen for larval provision. Nevertheless, they are clearly the primary cross-pollination agents for all *Krameria* species studied. The anthers of *Krameria* flowers (Fig. 1a, a) are clustered around the slightly longer style. When the bee alights, her body first contacts the stigma and then the masses of pollen extruding from the terminal anther pores. Some of this pollen is deposited on the underside of her body (usually on the hairs of the ventral portion of the thorax, the basal portions of the mid and hind legs or on a medial tuft of hairs on the second abdominal sternite) from which it can easily be transferred to the stigma of the next flower visited. The high floral constancy observed among all oil-foraging *Centris* presumably contributes to their efficiency as pollinators. *Krameria* species which we have tested seem to be self-incompatible and require cross-pollination.

The utilisation of a diet very rich in lipids by the larvae of a group of insects generally considered to depend on nectar and pollen (a high carbohydrate, low lipid diet^{5,6}) underscores the ignorance of the nutritional requirements and metabolic capabilities of the most important single group of angiosperm pollinators, the bees. Studies such as ours on oil-producing plants and their pollinators and the chemical nature of the oils may thus reveal not only novel

kinds of lipids but new hymenopteran metabolic pathways as well.

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Mating advantage of rare males in models of sexual selection

MODELS of sexual selection in polygynous species of animals have been derived on the assumption that some females have preferences to mate with males with particular genotypes^{1–3}. The mating advantage gained by the males is always frequency-dependent because the preferred males take part in the same number of preferential matings when they are rare as when they are common; individually therefore, they mate more often when they are rare. Frequency-dependent sexual selection has been demonstrated in many experiments with *Drosophila*: rare males take part in a higher proportion of matings than their frequency as available mates^{4–12}. Ehrman and Spiess¹⁰ explained this phenomenon by frequency-dependence either in female preference or in male courtship. This explanation, which is difficult to interpret in behavioural terms, may not be necessary, however, because constant female preferences would entail frequency-dependent selection among the males. I show here that a simple model of constant preferences for particular phenotypes or genotypes is sufficient to explain a large body of data on frequency-dependent sexual selection in *Drosophila*.

In models analysed previously^{1–3}, some females had mating preferences either for particular genotypes or for a phenotype determined by a dominant allele; other females mated at random. These possibilities can be combined in a model that allows for any degree of dominance as well as separate

Table 1 Fitting data of Spiess and Spiess to the model with constant mating preferences

(a) Matings with karyotypes homozygous for inversions AR and PP in *Drosophila pseudoobscura*^a

Before fitting the model $\chi^2 = 67.105$
 Maximum likelihood estimates: preference for AR, $\alpha = 0.138$
 preference for PP, $\theta = 0.021$

Ratio among males		Observed nos of matings of males		Expected nos of matings of males		Contribution to χ^2 after fitting
AR	PP	AR	PP	AR	PP	
2	18	51	214	58.856	206.144	1.348
4	16	63	135	60.627	137.373	0.134
6	14	38	60	38.249	59.751	0.003
8	12	56	43	46.965	52.035	3.307
10	10	49	37	48.031	37.969	0.044
12	8	69	37	68.115	37.885	0.032
14	6	78	19	70.489	26.511	2.928
16	4	182	41	180.807	42.193	0.042
18	2	292	40	297.104	34.896	0.834

After fitting the model $\chi^2 = 8.672$
 This value of χ^2 for 7 degrees of freedom shows there is no residual heterogeneity after the fitting of the model, the two parameters contributing $\chi^2 = 58.433$ to the overall χ^2 .

(b) Matings with strains Humboldt (Hu) and white wolf (WW) in *Drosophila persimilis*^a

Before fitting the model $\chi^2 = 101.707$
 Maximum likelihood estimates: preference for Hu $\alpha = 0.166$
 preference for WW $\theta = 0.156$

Ratio among males		Observed nos of matings of males		Expected nos of matings of males		Contribution to χ^2 after fitting
Hu	WW	Hu	WW	Hu	WW	
2	18	53	194	57.656	189.344	0.491
4	16	25	34	17.776	41.224	4.201
6	14	17	28	16.612	28.388	0.014
8	12	18	17	15.296	19.704	0.849
10	10	25	25	25.245	24.755	0.005
12	8	15	20	20.047	14.953	2.974
14	6	24	13	23.704	13.296	0.010
16	4	32	23	38.968	16.032	4.275
18	2	175	43	169.251	48.749	0.873

After fitting the model $\chi^2 = 13.692$
 This value of χ^2 shows no residual heterogeneity, the two parameters contributing $\chi^2 = 88.015$ to the overall χ^2 .

In the data of this table the matings of the two male genotypes are recorded in relation to the genotypes of females which were in a constant ratio of 50% while the males' frequencies were varied. Although PP and WW females mated less frequently than AR and Hu females, there is no evidence for assortative mating preferences in the two experiments and no heterogeneity in the relative frequencies of the matings of the males with the two female genotypes. In this table, therefore, the numbers of matings with the two genotypes of females have been added together.

preferences for each genotype. The preferences can be defined as follows:

genotypes	AA	AA'	A'A'
frequencies of genotype in population	u	v	w
proportions of females preferring each genotype separately	α'	β'	γ'
proportions of females preferring 'A' phenotypes	$au/(u+v) \quad av/(u+v) \quad -$		

It can then be shown¹³ that in the following generation the genotypic frequencies become

$$\begin{aligned} u' &= \phi p + \alpha p^2/(1-w) + p^2(1-\alpha-\theta) \\ v' &= \phi q + (\theta-\phi)p + \alpha p(2q-w)/(1-w) + 2pq(1-\alpha-\theta) \\ w' &= (\theta-\phi)q + \alpha q(q-w)/(1-w) + q^2(1-\alpha-\theta) \end{aligned}$$

where $p = u + \frac{1}{2}v$ and $q = w + \frac{1}{2}v$ are the gene frequencies of A and A'; and $\phi = \alpha' + \frac{1}{2}\beta'$, $\theta = \alpha' + \beta' + \gamma'$. Thus $\alpha + \theta$ is the total proportion of all females who mate preferentially; the remaining proportion $1 - \alpha - \theta$ mate at random. A stable polymorphic equilibrium is established at a gene frequency of A'

$$q_* = \frac{-\phi + \sqrt{(\phi^2 + 4(\theta-\phi)(\alpha+\theta))}}{2(\alpha+\theta)} \quad (1)$$

For a change of gene frequency from q_0 to q_n over a period of n generations, approximately

$$\left\{ \frac{q_n - q_*}{q_0 - q_*} \right\}^{(q_* + 1)} \left\{ \frac{q_n + q_* + a}{q_0 + q_* + a} \right\}^{(q_* + a - 1)} = \exp[-n(q_* + a)(\alpha + \theta)] \quad (2)$$

where $a = \phi/(\alpha + \theta)$.

Two cases are likely to apply to sexual selection in natural populations. When $\phi = \theta = \alpha'$ and $\beta' = \gamma' = 0$, A is at an unconditional advantage and spreads through the population to complete fixation. Among the females, a proportion α are more responsive to both AA and AA' males and a proportion ϕ are more responsive only to AA males. Thus in its effect on mating preference A is being selected as a semi-dominant or semi-recessive allele depending on the magnitudes of ϕ and α . In addition, some females may prefer A'A' males. Then $\phi = \alpha'$ and $\theta = \alpha' + \gamma'$ where γ' is the proportion of females preferring A'A' males. This produces a stable polymorphism at an equilibrium frequency given by equation (1). When A is completely dominant $\phi = \alpha' = 0$ and

$$q_* = \sqrt{(\theta/(\alpha + \theta))} \quad (3)$$

In this case $a = 0$ and the change in gene frequency over a period of n generations becomes

$$\begin{aligned} \left\{ \frac{q_n - q_*}{q_0 - q_*} \right\}^{(q_* + 1)} \left\{ \frac{q_n + q_*}{q_0 + q_*} \right\}^{(q_* - 1)} \\ = \exp[-nq_*(\alpha + \theta)] \quad (4) \end{aligned}$$

These models explain the establishment of stable polymorphisms in population cages of *Drosophila*. Equation (4) could be used to estimate the values of q_* and the total preference, $\alpha + \theta$. Combined with direct estimates of α and θ from observations of the numbers of the different matings, this would provide an independent test of the model.

If observations have been made of the numbers of matings for a range of different frequencies, the estimates of α and θ

can also be tested for heterogeneity against the null hypothesis that the proportions of females with preferences, α and θ , remain constant and determine the numbers of matings at each different frequency of the male genotypes. This test can be applied to Ehrman's and Spiess' data of number of matings of males at varying frequencies. Males of two different genotypes were used in the experiments. Females were introduced into the mating chamber containing the males and the genotype of the mated male was recorded. According to the model, the expectations are as follows:

genotypes	AA	BB
frequencies of genotypes of males	u	v
proportions of females preferring each genotype	α	θ
proportions of matings with each genotype	$\alpha + u(1 - \alpha - \theta) \quad \theta + v(1 - \alpha - \theta)$	

Suppose, in the i th experiment a_i and b_i are the numbers of matings observed with A and B males at frequencies u_i and $v_i = 1 - u_i$, then the log likelihood for all of k experiments is

$$\log_e L = \sum_{i=1}^k a_i \log[\alpha + u_i(1 - \alpha - \theta)] + \sum_{i=1}^k b_i \log[\theta + v_i(1 - \alpha - \theta)]$$

Maximum likelihood estimates of α and θ are then obtained as the values which maximise $\log_e L$. I have calculated these estimates by trial and error, starting with arbitrarily chosen values of α and θ and introducing random perturbations in the initial values until higher values of $\log_e L$ are found. By making the maximum size of the random perturbations smaller and smaller, the maximum of the log likelihood surface is reached by a random walk of successively smaller steps.

In Ehrman's experiments^{5, 6, 11}, the frequency of the female genotypes varied with the frequency of the male genotypes. Spiess^{8, 9}, however, kept the frequencies constant among the individuals who were being offered the choice of mates. Choice was generally exercised only by the females, the males mating at random when offered a choice of female genotypes. In his experiments on female choice, Spiess varied the frequencies of the male genotypes between experiments while maintaining the frequencies of the genotypes at 50% in the females. Thus, if choice varied according to genotype, this could not be a cause of the frequency-dependent advantage gained by the males in Spiess' experiments. Table 1 shows the data Spiess obtained and the fit of the model. The model provides a satisfactory fit with no residual heterogeneity after the expected numbers of matings have been calculated using the maximum likelihood estimates of α and θ from the total data of each of the experiments.

Table 2 shows the results of fitting Ehrman's data⁵ on the effect of frequency on the matings of males homozygous for the karyotypes CH and AR in *Drosophila pseudoobscura*. In these

Table 2 Fitting data of Ehrman⁵ on matings with karyotypes homozygous for inversions CH and AR in *Drosophila pseudoobscura* to the model with constant mating preferences

(a) Matings in a line that had been selected for positive geotaxis in previous generations						
All matings			Early matings			
Ratio among males	Observed nos of matings	Contribution to χ^2 after fitting	Observed nos of matings		Contribution to χ^2 after fitting	
CH	AR		CH	AR		
18	2	3.105	31	25	1.816	
16	4	0.140	36	24	0.025	
14	6	2.000	37	25	0.809	
12	8	2.662	21	33	2.198	
10	10	0.887	23	30	0.007	
8	12	9.645	31	23	7.731	
6	14	3.452	26	28	4.857	
4	16	0.070	14	45	0.778	
2	18	8.499	7	48	3.791	
Preference for CH		$\alpha = 0.217$			$\alpha = 0.189$	
Preference for AR		$\theta = 0.328$			$\theta = 0.310$	
Deviation from $\alpha = \theta = 0$		$\chi^2_2 = 213.591$			$\chi^2_2 = 98.014$	
Residual heterogeneity		$\chi^2_7 = 30.460$			$\chi^2_7 = 22.013$	
There is significant residual heterogeneity for the matings with male ratios 8:12 and 2:18. There are significantly too many matings of the less common males at the ratio 8:12 and too few matings of the less common males at the ratio 2:18. Otherwise, the fit of the model is very good with no consistent deviations from the model.						

(b) Matings in a line that had been selected for negative geotaxis in previous generations						
All matings			Early matings			
Ratio among males	Observed nos of matings	Contribution to χ^2 after fitting	Observed nos of matings		Contribution to χ^2 after fitting	
CH	AR		CH	AR		
18	2	3.089	45	9	2.728	
16	4	0.208	39	20	0.062	
14	6	0.039	36	20	0.143	
12	8	12.461	24	36	6.268	
10	10	1.122	26	32	0.683	
8	12	1.044	28	35	0.000	
6	14	0.720	21	35	0.033	
4	16	3.356	24	32	2.517	
2	18	0.005	16	43	0.000	
Preference for CH		$\alpha = 0.243$			$\alpha = 0.213$	
Preference for AR		$\theta = 0.292$			$\theta = 0.208$	
Deviation from $\alpha = \theta = 0$		$\chi^2_2 = 148.036$			$\chi^2_2 = 48.361$	
Residual heterogeneity		$\chi^2_7 = 22.044$			$\chi^2_7 = 12.435$	
There is still some residual heterogeneity among all matings after the fit of the model. This heterogeneity is determined by the high frequency of matings of the less common males at the ratio of 12:8.						

experiments, data at some frequencies show significant deviations from expectation after the fitting of the model: the ratios of 8:12 in the positively selected line and 12:8 in the negatively selected line show a great excess of matings of the less common males. No simple model would resolve these anomalies because at these ratios the mating advantage shows no particular relationship, frequency-dependent or not, with the mating advantages at the other ratios: there are no consistent deviations from the model. If preferences were genetically determined, chance would produce sampling variation in the proportions of females expressing a preference. If α and θ do vary between experiments, then the variance in the observed numbers of matings will be greater than the binomial variance. This will therefore inflate the values of χ^2 which have been calculated on the assumption of binomial variation. I consider this to be the most likely cause of the statistically significant heterogeneity in Ehrman's data. A model of the expected variance in α and θ would have to be constructed in order to calculate a corrected value of χ^2 .

Ehrman's experiment¹¹ on frequency-dependent mating for two recessive alleles *or* (orange) and *pr* (purple) also shows residual heterogeneity that is just statistically significant, and also without any systematic deviations from the model. In this experiment population cages were started at ratios of 80 *or*: 20 *pr* and 20 *or*: 80 *pr* and continued for 10 generations. In each generation the actual matings of 100 pairs were observed. It is to be expected that the mating preferences would vary between generations mainly as a result of sampling genetic drift. The variance in the observed numbers of matings must be greater than the binomial and χ^2 will therefore be inflated. Fitting the model to the data gives $\alpha = 0.244$ for the preference for *or* and $\theta = 0.259$ for the preference for *pr*. Before fitting the parameters, $\chi^2_{20} = 64.132$; and after fitting, $\chi^2_{18} = 30.823$ ($0.05 > P > 0.02$).

Ehrman⁷ tried to show "how a *Drosophila* female knows which males are rare". On my theory, the females do not have to carry out the difficult task of discriminating between rare and common males: constant mating preferences are sufficient to explain the observed frequency-dependence. In some of Ehrman's experiments, the mating advantage disappeared if the rare males in the mating chamber were the common males in an adjacent chamber from which air was passed into the mating chamber. This was interpreted to mean that olfactory stimuli from the adjacent chamber where the males were common prevented the discrimination of rare males in the mating chamber. The results, however, were "inconsistent and ambiguous" in some of these experiments. Sampling variation in the proportions of females expressing preferences might be the cause of both this inconsistency and the residual heterogeneity after fitting the data of some of Ehrman's experiments to my model.

O'Donald¹³ discussed the possible behavioural mechanisms which might give rise to mating preference. Some females may have a lower threshold of response to some male genotypes and would require less stimulation to mate with them. They would thus mate more readily with the preferred males and more readily than the other females who mate at random. The presence of other courting males will raise the general level of stimulation so that all females are stimulated to mate more readily. A female with a lower threshold towards a particular genotype of male would then mate more readily with any male. The proportion of preferential matings would therefore decline with increasing density. The mating preference, according to this model, will be density-dependent but not frequency-dependent. The disappearance of mating choice in some of Ehrman's experiments could be the results of the increased level of stimulation provided by the extra males in the adjacent chamber. A similar result would follow if the different male genotypes determined different levels of courtship activity. A more responsive female would be sufficiently stimulated by any male and so would mate more or less at random. A less responsive female would mate more readily with one of the more active males and would therefore tend to show a preference

that decreased at higher levels of stimulation. The mating preferences could thus be determined by variation either in male courtship or in female response. Both types of behavioural mechanism would explain observations of mating choice in *Drosophila*.

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Spontaneous forward mutation versus reversion frequencies for maize *Adh1* in pollen

RELIABLE quantitative data on spontaneous, specific gene mutation frequencies in higher plants and animals are few. Detergents include low natural frequencies and difficulty in obtaining in excess of 10^6 scorable organisms or gametophytes. The *alcohol dehydrogenase-1* gene (*Adh1* gene; ADH enzyme EC 1.1.1.1.), as expressed in pollen grains, is among the exceptionally suitable; much is known about the maize ADHs¹⁻³, *Adh1* function is totally dispensable in an aerobic environment and maize pollen is a trinucleate gametophyte which expresses much of its haploid genome, including *Adh1*^{4,5}. In particular, there have been two recent methodological advances. First, I am able to cytochemically stain pollen, before⁶ or after *in vitro* germination, for the presence of above 5% normal ADH activity. And second, ADH⁻ pollen grains survive allyl alcohol (C=C-C-OH) vapour concentrations which kill ADH⁺ grains^{6,7}; this selection scheme was developed for yeast by Megnet⁸. My genetic resolution is approximately one mutant (*Adh1*⁺→ADH⁻) per 10^7 chemically selected, viable gametophytes, and one (phenotypic) revertant (*Adh1*⁻→ADH⁺) per 10^8 unselected gametophytes. In this note, I compare spontaneous forward mutant frequency with previously published⁹ revertant frequencies for one naturally occurring and six ethyl methanesulphonate-induced *Adh1*-deficient (*Adh1*⁻) alleles.

About 2% of normal, *Adh1*⁺ pollen grains stain poorly in the ADH-specific reagents, a condition which almost always denotes inviability. For that reason, it was necessary to count ADH⁻ gametophytes among viable pollen rather than among total pollen. My methods were developed by using *Adh1*⁺/*Adh1*⁻ (*Adh1*- γ 25; CRM⁻; unpublished) heterozygotic plants segregating 1:1 for blue, opaque:yellow, translucent pollen. Pollen was collected under controlled conditions, cold treated, exposed to a series of allyl alcohol vapour concentrations, germinated on a slice from 'David's Bread Loaf' medium and subsequently frozen. The ice-perforated grains were then stained for ADH activity (methods described in Fig. 1 legend). Figure 1 shows the final result for an allyl alcohol concentration which kills 99.999% ADH⁺ pollen without affecting the viability of the 50% ADH⁻ grains: none of the blue, opaque grains have pollen tubes.

Wild-type *Adh1*⁺ pollen (either *Adh1*-S or *Adh1*-F isoalleles) was collected in an isolation greenhouse and treated according to the protocol outlined above. Allyl alcohol

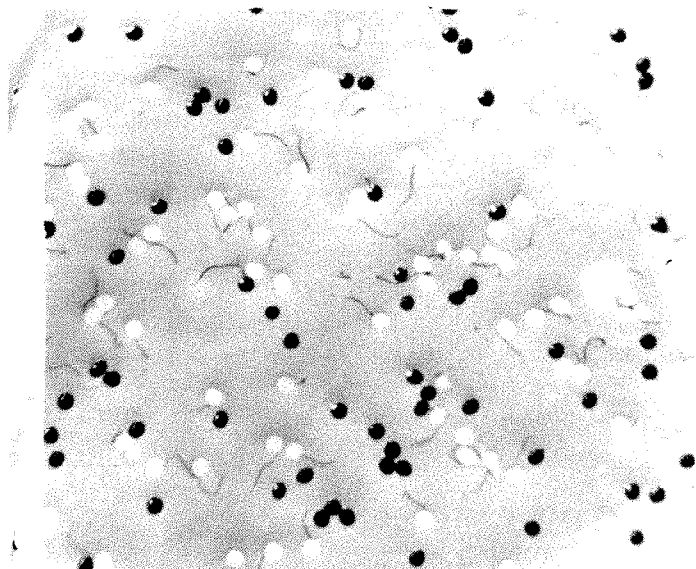


Fig. 1 Pollen shed from an *Adh1*⁺/*Adh1*⁻ plant after allyl alcohol treatment, *in vitro* germination, and *in situ* staining for ADH activity. Pollen was collected before 10 a.m., weighed, stored desiccated (CaSO₄) at 4 °C for 3 h and treated with 300 µM allyl alcohol for 30 min in a 500 ml sealed Mason jar. The treated pollen was brushed on to 2 mm slices from 'David's Bread Loaf' medium⁷, much modified from that of Cook and Walden¹¹, and allowed to germinate for 45 min. The slabs were frozen at -23 °C for 3 h and defrosted 30 min at 25 °C. ADH-specific stain was layered over the gametophytes three times, each for 30 min as previously described⁵. ADH⁺ gametophytes stained blue and opaque.

concentrations were chosen which allow a few ADH⁺ gametophytes to germinate. A forward mutant (*Adh1*⁺→ADH⁻) would be a yellow, translucent pollen grain with tube (allyl alcohol resistant) surrounded by blue, opaque non-germinated grains. Since pollen is naploid, ADH⁻ mutants might include modifiers. As shown in Table 1,

there were zero viable ADH⁻ grains among 4.9×10^6 viable gametophytes. The reliability of these data is supported by the following two considerations. (1) That allyl alcohol does not appreciably affect the viability of ADH⁻ pollen grains was insured by using borderline concentrations where some ADH⁺ gametophytes germinate; previous experiments⁷ indicated that the allyl alcohol concentrations I used killed no more than 20% ADH⁻ grains. (2) As a further control, I scored pollen shed from wild-type plants after the immature tassel containing the pollen mother cells was X-irradiated (last line, Table 1). Since the radiation-induced mutant frequency was rather high, these methods would have detected ADH⁻ gametophytes if they had occurred (*Adh1*⁺→ADH⁻ in mutants/viable gametophyte) at a frequency much above 2×10^{-7} .

My previous work⁵ quantified the (phenotypic) revertant frequencies (ADH⁺ revertants/unselected gametophyte) for one naturally occurring and six ethylmethane sulphonate-induced *Adh1*⁻ mutants. These mutants, recovered and characterised by Schwartz (see ref. 5) were chosen at random; two are CRM⁻ while the remaining five have lesions involving the structural gene component of the cistron. Five mutants are from the same inbred line³. A 'revertant' includes all mutants capable of changing an *Adh1*⁻ (yellow, translucent) to an ADH⁺ grain (blue; opaque): true revertants, second site revertants, unlinked suppressors; supersuppressors and overproducers are also possible for some mutants. An interesting mode of reversion would be the 'derepression' of the *Adh2* gene which is generally switched off in pollen; although possible, this explanation for high revertant frequency is not likely⁸. Reversion involving altered metabolic circuitry is not a factor since I measure *in situ* enzyme activity directly. The revertant frequencies $\pm 1 \text{ s.e.} \times 10^6$ were 1.6 ± 0.4 ; 7.3 ± 1.4 ; 2.1 ± 1.0 ; 7.2 ± 1.1 ; 10.8 ± 1.7 ; 1.2 ± 0.3 and 5.7 in ADH⁺ per gametophyte. The median revertant frequency was 5.7×10^{-6} ADH⁺ per gametophyte. This frequency is an underestimate, since 5% normal ADH activity and perhaps as

Table 1 Spontaneous *Adh1*⁺ to ADH⁻ mutant frequency

F ₂ <i>Adh1</i> Genotype	Control % Germination	Grains $\times 10^{-5}$ (Total)*	(Viable)	C=C-C-OH (µM)	No. (or %) germination† Post-selection†	No. ADH ⁻
S/F (12 plants)	76.25	4.00	3.05	222	0	0
	72.50	4.00	2.90	222	0	0
	92.50	4.00	3.70	222	0	0
	83.00	5.00	4.15	222	0	0
	70‡	4.00	2.80‡	37	0	0
	70‡	3.00	2.10‡	37	0	0
	76.00	3.60	2.74	15	23	0
	90.00	1.60	1.44	15	4	0
	86.25	2.00	1.73	15	18	0
	84.50	3.40	2.87	6	(41.5)	0
	86.30	2.92	2.52	15	(32.0)	0
	88.00	3.00	2.64	15	(16.3)	0
F/F (4 plants)	79.00	4.00	3.16	222	0	0
	70‡	2.14	1.50‡	37	0	0
	70.00	4.00	2.80	37	0	0
	76.7	3.00	2.30	7§	(12.0)	0
S/S (3 plants)	70‡	2.55	1.78‡	37	0	0
	93.60	1.60	1.50	15	(40.0)	0
	82.50	3.72	3.07	6§	(73.5)	0
		Total	48.75			0
S/S (5 plants) (X-rayed)	45.0 (incl. 40% abortion)	6.64	2.99	148	57	19 (or 6.4×10^{-6} mutants per viable gametophyte)

* No. grains calculated on the basis of control experiments indicating that 1 mg fresh pollen of these families equals 2,000 grains. The X-irradiated plants gave 40% aborted pollen; we assumed that weight of an abortant was 10% that of a plump grain.

† Every allyl alcohol survivor was stained in hopes of finding an ADH⁻.

‡ Minimum values.

§ Treated for 25 rather than 30 min.

|| 720 rad at 56 rad min⁻¹ were delivered to the pollen mother cells. Undoubtedly, some of the germ cells were premeiotic while others were past microspore mitoses; these data are not to be quantified.

Table 2 Paired data on spontaneous forward mutation versus median reversion frequencies for specific genes

Organism	Cistron(s)	f (forward mutation)	Units	Ref.	Median f (reversion)	Units	No. alleles	Ref.	f (reversion) / f (forward mutation)
T-4	<i>RIIA</i> and <i>RIIB</i>	4.5×10^{-4}	75% r/colony	12	$5 \times 10^{-8*}$	revertants/viable phage	20	13	10^{-4}
<i>N. crassa</i>	<i>Ad-3A</i> and <i>Ad-3B</i>	3.8×10^{-7}	mutants/viable conidium	14	$2 \times 10^{-6*}$	revertants/viable conidium	4†	15	5.3
Maize	<i>wx</i>	$<7 \times 10^{-7}$	mutants/seed	16	7×10^{-6}	revertants/pollen	14	17	>10
	<i>Adh1</i>	$<2 \times 10^{-7}$	mutants/viable pollen	—	5.7×10^{-6}	revertants/pollen	7	—	>29
Mouse	<i>a, b, c, d,</i> and <i>In</i> pooled	$1.3 \times 10^{-5}\ddagger$	mutants/locus/mouse	18	$2.7 \times 10^{-6*}$	revertants/locus/mouse	5§	18	0.2

* All were either true revertants or other intragenic events.

† Of 24 X-ray induced mutants, only these four reverted at frequencies above 2×10^{-8} .

‡ Macroleisions pass through the gametes.

§ One allele for each of these five loci; only *a* and *d* gave revertants where f s below 2×10^{-6} per locus per mouse go undetected.

|| Auerbach and Kilbey¹⁹ commented: "Surprisingly, forward and reverse mutation rates at five of these loci differed only by a factor of three."

high as 30% normal ADH activity/gametophyte is scored as ADH⁻ (ref. 5). Recovery and analysis of these revertants is most important and is underway. In any case, $5.7 \times 10^{-6} / <2 \times 10^{-7}$ gives a >29-fold excess of spontaneous revertant over forward mutant frequency.

The comparison of forward mutant and revertant frequencies for specific genes is fraught with methodological pitfalls, hidden assumptions and statistics of dubious applicability. Table 2 compiles some comparative data for a few genes in a few organisms. These data comprise the totality of reliable data found—no selection was intended. Some of the more apparent considerations affecting the reliability of these data follow. Higher animals are expected to show inflated forward mutant frequencies in the absence of rigorous genetic analyses because there is no haploid (gametophytic) stage to impede the transmission of macrolesions. Ideally, a revertant frequency per gene should be based on the median frequency of several revertable alleles. Spontaneous reversion data for *Drosophila* is for 'unstable' alleles or for too few alleles (M. M. Green, personal communication; Green also notes that high reversion is expected of insertion-type mutants). Marker effects perhaps reflecting the mutagenic agent, the contribution of alternative metabolic pathways to reversion frequencies, meiotic effects, variation in modifiers of spontaneous mutagenesis, cell lineage differences between sexes, and differences in germ cell development are all known to affect mutation rates in certain cases. The fundamental nature of the cistron itself might also differ among different organisms^{9,10}. It is generally believed that forward mutant frequencies exceed median revertant frequencies, especially when suppressors are ruled out. But the data for the purple adenine mutants in *Neurospora* and five coat colour loci in mice (Table 2) do not fit this rule. Observations on red adenine mutants in yeast—the homologue of purple adenines in *Neurospora*—support the canonical view that spontaneous forward mutation exceeds median reversion frequencies (H. Roman, personal communication). The clear excess of revertant frequency over forward mutant frequency for the two cistrons studied in maize might be explained readily if almost all revertants were suppressors. However, for both *Adh1* and *wx* (Table 2), every mutant gave relatively high revertant frequencies; nonsense suppression is not likely.

The significance of our *Adh1* comparison and the few other results where spontaneous reversion exceeds forward mutation frequency cannot be assessed readily. It is clear that evolutionary and other arguments resting heavily on

the assumption that reversion frequencies are comparatively negligible should be reevaluated.

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Determinant selection is a macrophage dependent immune response gene function

IMMUNE response (Ir) genes are linked to the species histocompatibility complex and define as yet uncharacterised phenotypic products which control the immune response to thymus dependent antigens¹. Antibody formation² and antigen induced T lymphocyte proliferation³ are two examples of immune phenomena which, *in vivo* and *in vitro*, operate under Ir gene influence. To clarify their mechanism of action and cellular location, we have examined the contribution of antigen structure (amino acid sequence and conformation to Ir gene control of antigen recognition by T lymphocytes) as well as to the critical role played by the antigen presenting macrophage in expression of that control^{4–7}. We report that immune response gene control of antigen recognition operates at least in part at the level of the macrophage.

Recent studies of *in vitro* T-cell proliferation in insulin in inbred guinea pigs offered us a unique opportunity to examine, operationally, the site of Ir gene product function¹. T cells from both strain 2 and 13 guinea pigs immunised with pork insulin in complete Freund's adjuvant proliferate *in vitro* response to insulin. The determinant recognised by the strain 13 guinea pig resides in the B-chain region of the insulin molecule as evidenced by the capacity of insulin immune strain 13 but not strain 2 guinea pigs to respond to isolated oxidised B chain. When strain 2, 13 and F₁ (2×13) guinea pigs are immunised with oxidised B chain, strain 13 and F₁, but not strain 2, respond to either isolated B chain or native insulin. Genetic analysis of backcrossed and phenotypically characterised outbreds immunised to B chain showed that the relevant immune response gene defining response to the B chain determinant is linked to the strain 13 guinea pig histocompatibility complex. By contrast, the strain 2 inbred guinea pig T cell recognised insulin through a determinant present in insulin A chain. More specifically, they recognised the A chain loop and their T-cell response is sensitive to amino acid changes in position A8, A9 and A10. Insulins with total identity in this area completely cross-react in T cell proliferation and T helper cell activity. Insulins with partial or no identity in these amino acids residues show partial or no cross-reactivity respectively. In strain 13 animals, different species variants of insulin show cross-reactivity at a T-cell level independent of their A chain loop constitution. As anticipated, F₁ T cells from insulin immune guinea pigs are able to recognise either determinant.

We assessed the function of macrophages in Ir control taking advantage of the capacity of T cells from insulin immunised F₁ (2×13) guinea pigs to respond to antigen bearing parental macrophages. We reasoned as follows.

If Ir gene function operates exclusively at the level of the responding lymphocyte, both determinants should be simultaneously recognised by F₁ T cells independently of which parental macrophage (Mφ) is 'presenting' the antigen. Conversely, if the definition of which determinant is actually recognised depends on the genetic profile of the antigen presenting Mφ, then Ir gene function must also be operating at this cell level. The data that follow favour the latter assumption. Mφ from both inbred strains of guinea pig are able to 'present' pork insulin to pork insulin immune F₁ (2×13) lymphocytes (Table 1). As with PPD, a strong proliferative response is observed when pork insulin pulsed Mφ from either strain of guinea pig are added to F₁ PELs. When different species variants of intact insulin are presented to the same pork insulin immune F₁ PELs by either strain 2 or 13 Mφ, however, two different patterns of response are seen (Fig. 1). An A-chain loop pattern of cross-reactivity is observed when the antigens are presented on

Table 1 DNA synthetic responses of T cells from pork insulin immunised F₁ (2×13) guinea pigs to parental macrophages pulsed with native insulin

Macrophages pulsed with:	Macrophage genetic background	
	Strain 2	Strain 13
	³ H-TdR incorporation Δc.p.m. × 10 ⁻³	
PPD	197.45 ± 6.60	175.04 ± 29.91
Pork insulin	45.51 ± 11.34	62.42 ± 10.59

F₁ (2×13) guinea pigs were immunised with 100 μg of pork insulin in CFA, 2–3 weeks before use. Oil induced, macrophage-rich peritoneal exudate cells were pulsed with 100 μg ml⁻¹ antigen and 40 μg ml⁻¹ of mitomycin C at 37 °C for 60 min. After four washes 1.2 × 10⁶ of these cells were added to 2.4 × 10⁶ responding peritoneal exudate lymphocytes depleted of adherent cells (PELs) in 200 μl of 5% heat inactivated male guinea pig serum in RPMI-1640 plus 2.5 × 10⁻⁵ M of 2-mercaptoethanol in round microtitre plates. After 48 h of culture 1 μCi of ³H-methyl-thymidine (6.7 Ci mmol⁻¹) was added for an additional 24 h. Cells were collected on glass fibre filters with a semi-automated microharvester (Adaps) and ³H-thymidine incorporation determined by liquid scintillation spectrometry. Data are mean ± s.e.m. from four experiments. PPD, purified protein derivative of tuberculin.

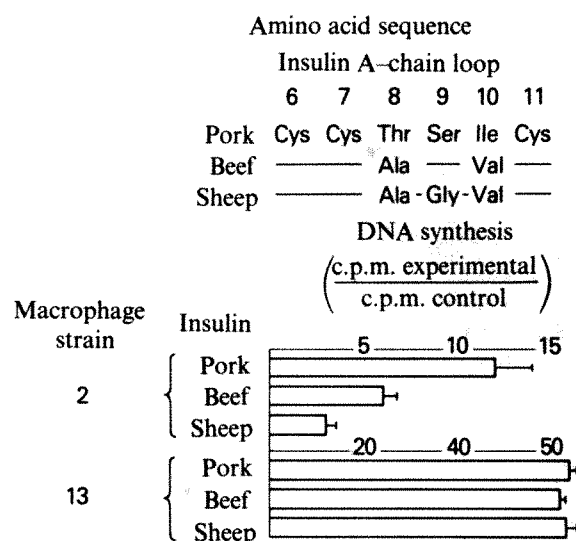


Fig. 1 Different response patterns observed in F₁ (2×13) guinea pig pork immune T cells when the antigen are 'presented' by strain 2 or strain 13 macrophages. In an experimental protocol similar to that described in Table 1 pork insulin immune F₁ (2×13) T cells were challenged 'in vitro' by strain 2 and strain 13 Mφ pulsed with pork, beef and sheep insulins. When the different insulins are presented by strain 2 Mφ, there was a good proliferative response to pork insulin, an intermediate response to beef insulin and almost no response to sheep insulin. There was a substantial proliferative response to the three different insulins when presented on strain 13 Mφ (b). Beef and pork insulins share one identity in the insulin A-chain loop and pork and sheep share no amino acids identities in this area (a). The amino acid sequence of the remainder of the beef, pork and sheep insulin molecule is identical.

strain 2 Mφ. This pattern of cross-reactivity is characterised by partial cross-reactivity between pork and beef insulin (partial identity in the loop) and little cross-reactivity between pork and sheep insulin (no identities in the loop). To corroborate the above findings and to show that a specific B-chain determinant is being selected when the whole insulin molecule is presented by strain 13 Mφ, we studied the response of oxidised insulin B chain immune F₁ (2×13) PELs to parental Mφ pulsed with native insulin or isolated B chain. Only strain 13 Mφ can present native insulin to B chain immune F₁ (2×13) PELs (Table 2). Despite the capacity of strain 2 Mφ to present the same insulin to insulin

Table 2 DNA synthetic response of T cells from oxidised B chain immunised F₁ (2×13) guinea pigs to parental macrophages pulsed with native insulin and isolated B chain

Macrophages pulsed with:	Macrophage genetic background	
	Strain 2	Strain 13
	³ H-TdR incorporation Δc.p.m. × 10 ⁻³	
PPD	145.81 ± 11.32	98.01 ± 14.10
Pork insulin	0.10 ± 0.09	13.16 ± 3.09
Oxidised B chain	6.84 ± 3.74	66.90 ± 19.60

Experimental procedures were as for Table 1 except F₁ (2×13) guinea pigs were immunised with 10 μg of oxidised insulin B chain 2–3 weeks before use. Data are mean ± s.e. for five experiments.

immune F₁ T cells, they cannot initiate DNA synthesis in B-chain immune F₁ T cells. Parenthetically, a considerable proliferative response is also seen when isolated insulin B chain is presented to F₁ T cells on strain 13 Mφ but less response is seen when the same antigen is bound to strain 2 Mφ.

Such studies raise the possibility that in F₁ (2×13) animals immunised to insulin, two distinct sets of clones of T cells are generated: one that recognises the α loop antigenic determinant 'processed' by or 'presented' by the strain 2 specific cell structures in the F₁ Mφ, and one that recognises

the B-chain determinant generated by the strain 13 counterpart of the system. This hypothesis has been examined by bromodeoxyuridine (BUdR) and light elimination experiments* in F₁ guinea pigs.

Initial culture of insulin bearing parental macrophages with insulin immune F₁ T cells in the presence of BUdR and light selectively decreases responsiveness, on subsequent exposure to insulin bearing macrophages identical to those originally used to elicit cell activation (Table 3). Thus, preculture of F₁ T cells with strain 2 macrophages bearing insulin significantly depresses (65%) their response to antigen on strain 2 macrophages on subsequent cultures without comparably affecting responsiveness to insulin presented by strain 13 macrophages. Conversely, exposure of F₁ T cells to strain 13 macrophages bearing pork insulin eliminates 87% of responsiveness on repeat culture to pork insulin on strain 13 macrophages but does not affect the response of F₁ T cells to strain 2 macrophages bearing pork insulin. The clone remaining after elimination of T-cell proliferation by strain 2 macrophages pulsed with pork insulin responds equally well to strain 13 Mφ pulsed with either sheep (32,010 c.p.m.) or pork insulin (36,530 c.p.m.). Sheep insulin is identical to pork insulin except for the three amino acids of the A-chain α loop. Correspondingly, after BUdR and light elimination by strain 13 macrophages bearing insulin, the remaining cells respond only to strain 2 Mφ bearing pork but not sheep insulin.

Although immune specificity is traditionally considered a lymphocyte function*, previous observations from ours and from other laboratories indicate that the macrophage has some discriminatory function in the antigen recognition process (refs 3, 7, 9, 10, 12, 14, 15, 17).

Table 3 BUdR and light elimination of antigen specific F₁ T-cell proliferation: intramolecular determinant selection by pork insulin-bearing parental macrophages

Macrophages used in 1st culture	% Elimination of F ₁ T-cell DNA synthetic response on 2nd culture	
	Macrophage used:	
	Strain 2	Strain 13
Strain 2	65 (25-96)*	7 (0-12)
Strain 13	0	87 (70-97)

Immune PELs at 1×10^6 ml⁻¹ were cultured with 3×10^5 mitomycin C treated insulin pulsed Mφ for 48 h in 12 × 75-mm capped plastic tubes. At the end of this time $2 \mu\text{g ml}^{-1}$ of freshly prepared BUdR was added to the cultures. Twenty-four hours later, the cell pellets were exposed to light by placing the culture tubes directly on an array of three fluorescent light bulbs (Cool-Ray GE) for 90 min. After four washes of the cell pellet with Hanks BSS, the BUdR and light-treated cells were cultured a second time in microtitre plates (as described in Table 1) in the presence of added pork insulin-pulsed Mφ. Data are expressed as % inhibition of DNA synthesis remaining after BUdR and light treatment. BUdR is a thymidine analogue which, if present during the S phase of the cell cycle, is incorporated into newly synthesised DNA and cross-links DNA strands upon light activation. Treatment of activated lymphocytes with BUdR and light leads to an irreversible block in cell replication.

*Values are averages of three experiments, with range in parentheses.

Although not excluding the possibility that Ir genes also function at the T-cell level, our data suggest that macrophages have a fundamental role in selecting, in a complex antigen, the moiety(ies) to be recognised by immune T cells. Indeed these experiments are of particular relevance in view of recent studies using BUdR and light to eliminate antigen specific T cells in populations of F₁ (2 × 13) lymphoid cells from guinea pigs immune to multi-determinant antigens¹⁸. Such experiments showed that elimination of T cell responsive to antigen associated with macrophages of one parental haplotype failed to eliminate responsiveness to the same antigen on the other parental macrophage. Those data were interpreted to indicate either: (1) that cells possess dual receptors, one for antigen and a second independent receptor for H-linked surface molecules or (2) that a

'compound antigen determinant' consisting of antigen in unique association with an Ia antigen not antigen alone, is recognised by a single T-cell receptor¹⁹. An additional interpretation raised by our studies of the immune response to insulin, is that a selected amino acid sequence and/or conformation within the antigen itself is seen by the T-cell receptor. Generation or display of such antigenic determinants would thus be a function of immune response genes operating at the level of the antigen presenting cell. Two general mechanisms by which macrophages might subserve such a function may be proposed. One possibility is that immune response genes define a class of receptors or broad specificity which recognise molecular shape and thus have the unique ability to focus or orient distinct regions of the antigen for presentation to the T cell. A second possibility is that immune response gene products are or regulate the activity of families of enzymes which modify or metabolise polypeptide antigens. In this latter situation, the repertoire of Ir genes associated with a given haplotype would define restricted areas of the molecule as available for display to the T-cell receptor. Experiments to distinguish these possibilities are in progress.

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Nucleoside-specific tolerance suppresses anti-nucleoside antibody forming cells

SINCE its original development by Jerne¹, the haemolytic plaque assay has increased our understanding of antibody formation to a wide variety of antigens, including proteins, lipopolysaccharides, and simple haptens². We have now developed an assay to detect plaque forming cells (PFC) making anti-nucleoside antibodies. Previously we reported the suppression of circulating antibody to DNA determinants by nucleoside-IgG conjugates^{3,4}. Here we show that BALB/c mice can be rendered tolerant in terms of both direct and indirect anti-nucleoside antibody forming cells and that the state of tolerance is nucleoside-specific at the cellular level.

Nucleosides were conjugated to BALB/c IgG2a (RPC₅) myeloma protein as tolerogen or to keyhole limpet haemocyanin (KLH) as immunogen by the procedure of Erlanger and Beiser⁵. Tolerogen preparations were (AGCT)₂₅-IgG2a and G₂₅-IgG2a and the immunogen preparation was (AGCT)₁₂₀-KLH; (the subscript numbers indicate the total molar ratio of hapten substitution on the

carrier, assuming 800,000 as the molecular weight of KLH).

To determine whether BALB/c mice immunised with 0.2 mg of AGCT-KLH in complete Freund's adjuvant intraperitoneally would produce antibody forming cells to AGCT-target sheep cells, groups of five mice were killed and assayed for direct and indirect PFC on various days after immunisation. The peak of direct anti-nucleoside PFC development occurred on days 6 and 7, and that of indirect

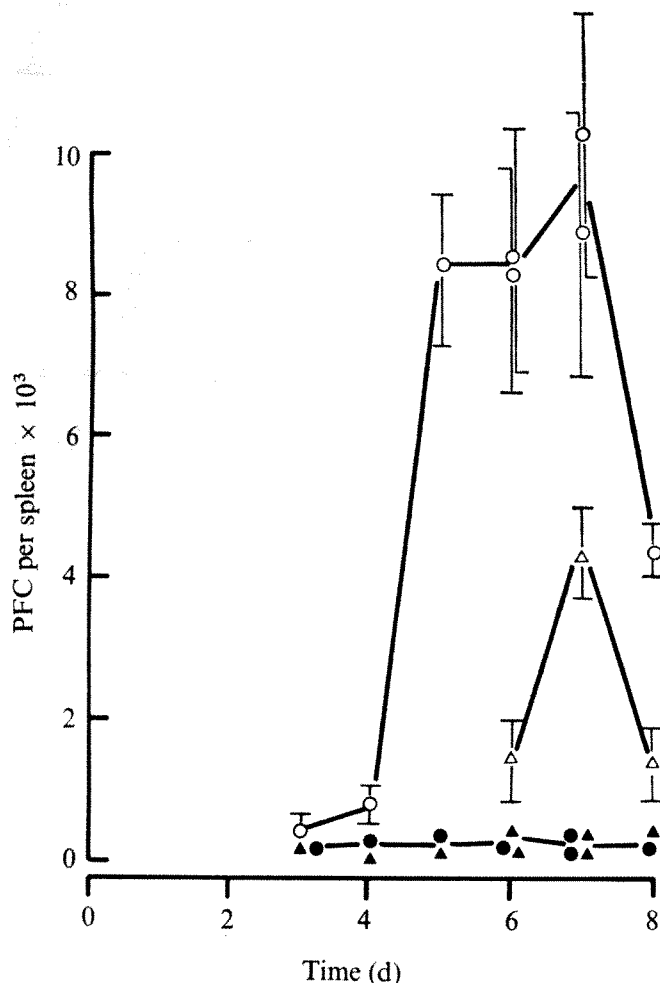


Fig. 1 Development of specific anti-nucleoside PFC, measured as the geometric mean ($\pm$ s.e.) PFC per spleen for groups of three non-immunised mice or five mice immunised with 0.2 mg of AGCT-KLH on day 0. Target cells were AGCT-SRBC. $\circ$, Direct and $\triangle$, indirect anti-nucleoside PFC in immunised mice. $\bullet$, Direct anti-nucleoside plaques in non-immunised mice. $\blacktriangle$, PFC to unsubstituted SRBC in immunised and non-immunised mice. The animals were 8–10 wk old BALB/c males (Jackson Laboratory). To prepare nucleoside-SRBC target cells, nucleosides were conjugated to the cells by the method of Erlanger and Beiser⁹ as modified to eliminate conditions that were damaging to the cells. A mixture of 5 mg of each nucleoside was oxidised in 1.5 ml of 0.1 M sodium periodate in 0.15 M NaHCO₃ for 20 min at room temperature; the reaction was stopped by the addition of 15 μ l of ethylene glycol. Sheep erythrocytes were washed twice with 0.15 M NaHCO₃ and 0.5 ml of packed cells were then suspended in 2.0 ml of the bicarbonate solution in a 40 ml centrifuge tube. The oxidised nucleosides were added dropwise to the cell suspension and the mixture was kept at room temperature for 15 min. 100 mg of *t*-butylamine borane (Aldrich) in 5 ml of 0.15 M NaHCO₃ was added. The suspension was kept at room temperature for 3 min and the tube was quickly filled with bicarbonate solution and centrifuged at 1,500 r.p.m. for 10 min. The modified cells were washed twice more with the bicarbonate solution and were ready for use as target cells in the haemolytic plaque assay of Jerne performed as before⁸. The indirect plaque forming cells were revealed by use of a rabbit anti-mouse IgG serum shown to react with IgG1, IgG2a, IgG2b, and IgG3 on immunodiffusion. The serum was used at a dilution of 1/200. IgG (7S) PFC were obtained by subtracting the direct PFC from the total number of PFC. Statistical analysis with Student's *t* test.

PFC on day 7 (Fig. 1). The spleens of non-immunised mice did not contain PFC to AGCT-SRBC spontaneously, and mice immunised with AGCT-KLH did not have a significant increase in the number of PFC to unsubstituted sheep red blood cells (SRBC).

To determine whether a nucleoside-IgG2a conjugate would suppress the appearance of anti-nucleoside antibody forming cells, mice were treated with 0.2 mg of tolerogen (AGCT-IgG2a) immediately before immunisation. They developed virtually no direct or indirect PFC (Fig. 2). Similar results were obtained when the tolerogen was given intravenously (i.v.) 10 d before immunisation (Table 1).

We then determined whether one can detect direct PFC to individual nucleosides in mice immunised with all four nucleosides, to allow a study of the nucleoside specificity of the tolerance. Seven BALB/c mice were injected with 0.2 mg of G-IgG2a. Ten days later, together with untreated controls, they were immunised intraperitoneally (i.p.) with 0.2 mg of AGCT-KLH in complete Freund's adjuvant, and the direct haemolytic plaque assay for all four nucleosides together (AGCT-SRBC), as well as for individual nucleoside SRBC targets, was performed 6 d later. Mice immunised with AGCT-KLH developed PFC to each of the four nucleosides (Table 2). The number of PFC was greatest for G and was much smaller for A than for any of the other nucleosides. Mice made tolerant by G-IgG2a had a specific suppression of antibody forming cells to this nucleoside only.

Several points are worth emphasis. First, when tolerance was induced in BALB/c mice by injection of nucleoside-IgG2a conjugates, the mechanism involved a reduction in the number of antibody forming cells and not simply the removal of preformed circulating antibody, suggesting that a similar mechanism may have occurred in (NZB/NZW)F1 mice made tolerant by nucleoside-IgG conjugates⁷. Second, when mice were given the G-IgG2a tolerogen and immunised with AGCT-KLH, they developed PFC to any nucleoside not present on the tolerance inducing carrier, demonstrating the exquisite specificity of tolerance at the cellular level. Third, tolerance was induced by nucleoside-IgG in terms of both direct and indirect PFC. It was also notable that while antibody forming cells to individual nucleosides could be detected readily in control mice

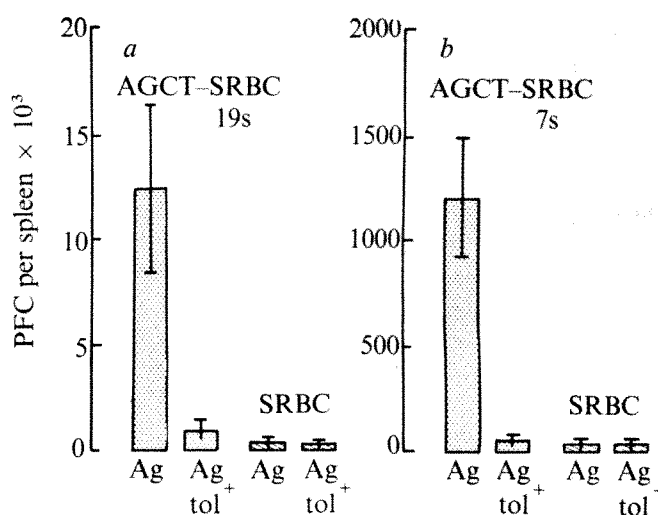


Fig. 2 Suppression of anti-nucleoside PFC formation by nucleoside-IgG conjugate. Each bar represents the geometric mean ($\pm$ s.e.) PFC per spleen of a group of five BALB/c mice either immunised i.p. with 0.2 mg of AGCT-KLH in complete Freund's adjuvant without pretreatment, or treated with 0.2 mg of AGCT-IgG2a immediately before the immunisation. Both AGCT-SRBC, prepared as described in the legend to Fig. 1, and unsubstituted SRBC were used as target cells in the haemolytic plaque assay done 6 d after immunisation. *a*, Direct PFC on day 6; *b*, indirect PFC on day 7.

Table 1 Effect of nucleoside-IgG2a conjugate on appearance of anti-nucleoside antibody forming cells

Treatment 10 d before immunisation	Direct (AGCT) PFC per spleen on day 7	<i>P</i>	Indirect (AGCT) PFC per spleen on day 7	<i>P</i>
None	10,173 ± 1,867		1,862 ± 594	
AGCT-IgG2a	1,888 ± 480	<0.001	498 ± 202	<0.01

Seven BALB/c mice were treated with 0.2 mg of AGCT-IgG2a, given i.v. and, together with seven untreated mice, were immunised i.p. with 0.2 mg of AGCT-KLH in complete Freund's adjuvant 10 d later. Direct and indirect PFC were measured on day 7, as described in the legend to Fig. 1. Values are means ± s.e.

Table 2 Nucleoside specificity of tolerance

Treatment 10 d before immunisation	AGCT-SRBC	<i>P</i>	Direct PFC per spleen on day 6	<i>P</i>	C-SRBC	T-SRBC
			A-SRBC			
None	19,970 (7,283)		1,055 ± 922		6,706 ± 3,730	7,943 ± 4,546
G-IgG2a	12,751 (7,283)	>0.4	1,535 ± 689	<0.01	5,824 ± 4,791	6,067 ± 2,066

Seven BALB/c mice were treated with 0.2 mg of G-IgG2a and, together with seven untreated mice, were immunised i.p. with 0.2 mg of AGCT-KLH in complete Freund's adjuvant 10 d later. Individual nucleoside-SRBC target cells were prepared as in the legend to Fig. 1, but in each case 10 mg of nucleoside were oxidised in 1.0 ml of 0.1M sodium periodate. Direct PFC were measured 6 d after immunisation. Values are means ± s.e.

immunised with all four nucleosides on the immunogenic carrier, there was some variation in the number of PFC specific for each nucleoside. This reflected at the cellular level of the immunodominance of guanosine and the low immunogenicity of adenosine that we have noted previously for serum antibody responses⁴. Finally, from a technical point of view, the modifications of the technique of Erlanger and Beiser⁵ to link nucleosides to sheep cells should be noted. The ionic strength had to be maintained to prevent lysis. The use of 0.15 M bicarbonate did this and provided a suitable pH for the Schiff base formation. The use of *t*-butylamine borane complex in place of sodium borohydride was important in preventing damage to the erythrocytes during reduction of the Schiff base, and the reduction time was brief.

The immune response to nucleic acid antigens is important from at least two points of view. First, since anti-nucleic acid antibodies are involved in autoimmune disease⁶, the suppression of this response can be of therapeutic importance; and second, strain differences in the ability of mice to respond to nucleic acid antigens have been observed⁹ and might offer the opportunity to study the genetic basis of both immunity and tolerance to defined determinants of naturally occurring antigens. The development of an assay that permits the detection of antibody forming cells to either all four nucleosides simultaneously or to individual nucleosides is useful for the study of these phenomena.

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T-cell cytotoxicity in the absence of viral protein synthesis in target cells

CYTOTOXIC T cells lyse only those virus infected target cells *in vitro* which express, in addition to the viral antigen(s), those *K* or *D* region products of the major histocompatibility complex (MHC) which were present during anti-viral sensitisation *in vivo*. This 'associative recogniton' by cytotoxic T cells could reflect the interaction of two T-cell receptors with specificity for target *K* or *D* gene products and independently for the viral antigen, or one receptor

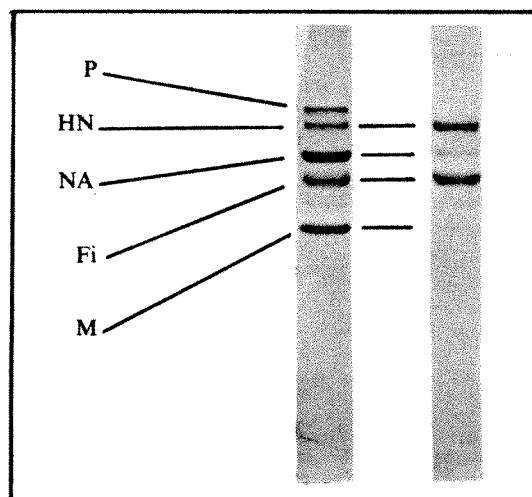


Fig. 1 Separation of Sendai virus envelope protein on SDS-polyacrylamide gels. Sendai virus (8 mg ml^{-1}) in phosphate-buffered saline without Mg or Ca (PBS-A), 1.5×10^6 haemagglutinating units (HAU) per ml were treated with 0.25% NP 40 for 15 min at 20°C (ref. 6). The suspension was centrifuged at $120,000g$ for 1 h at 4°C to remove partially disrupted virions and nucleocapsids. The supernatant was dialysed for 4 d at 4°C against twice daily changes of PBS-A using spectrophor 2 tubing (Raven Scientific). The reassembled membrane particles were concentrated by centrifugation at $120,000g$ for 1 h at 4°C and the pellet was resuspended in PBS-A. Envelopes were active in assays for haemagglutination⁷, neuraminidase⁸ and haemolytic activities⁹. Envelopes were analysed by SDS-polyacrylamide electrophoresis by the method of Laemmli¹⁰ using 10% gels. P, polymerase; HN, haemagglutinin; NA, Neuraminidase; Fi, fusion factor; M, M-protein.

with specificity for virally altered *K* or *D* region products (see ref. 1 and refs therein). There are various ways that the MHC antigens could be altered, including 'modification from within', where the virus modifies host protein synthesis by interfering with transcription², translation or post-translational glycosylation; or 'modification from without' where enzymic or chemical alteration of cell membrane proteins are induced by virus activity at the cell surface. In this report we show that inactivated Sendai virus or isolated Sendai virus envelopes can serve to modify a cell and make it a specific target for Sendai-immune T-cell killing, thus excluding the possibility of 'modification from within' in this system.

Sendai virus-primed BALB/c spleen cells, boosted *in vitro* with syngeneic infected blast cells, specifically killed Sendai virus-infected syngeneic target cells; primed spleen cells boosted with syngeneic non-infected cells did not (Table 1). Killer cells after secondary stimulation were T cells as shown by treatment with anti-Ly-sera (U. K. and P. Beverley, unpublished). Three approaches were used to show that synthesis of viral protein is not required in the target cell in order for it to be killed by virally sensitised T cells. First, cells incubated with ultraviolet-irradiated Sendai virus were lysed by sensitised T cells (data not shown). Second, cells exposed to purified Sendai virus, inactivated with β -propiolactone—which abolishes induction of protein synthesis by inactivated particles³—also proved to be effective target cells (Table 1). Treatment with β -propiolactone reduced the number of plaque forming units by 10^8 . Since the ratio of particles to plaque forming units would be expected to be no better than 10:1 (J. Skehel, personal communication) and target-to-particle ratio was 1:10⁶, it follows that, at most, one in a thousand cells were infected with a live virus. Sendai virus specific killer cells generated by secondary stimulation *in vitro* lyse only *H*-2-compatible infected target cells (U. K., unpublished) and the same restriction is seen using target cells incubated with inactivated Sendai virus (Table 2). Third, cells exposed to isolated envelopes from Sendai virus, which contained mainly the two surface glycoproteins with a very small amount of nucleocapsid and almost no detectable M-protein (Fig. 1), were susceptible to T cell-mediated lysis. To exclude nonspecific effects of the Sendai envelopes on target cells, influenza virus-specific cytotoxic T cells were tested on these target cells, and found to be ineffective (Table 3).

In several virus infections, target cell formation requires translation of early viral proteins¹¹⁻¹³, glycosylation¹⁴, and

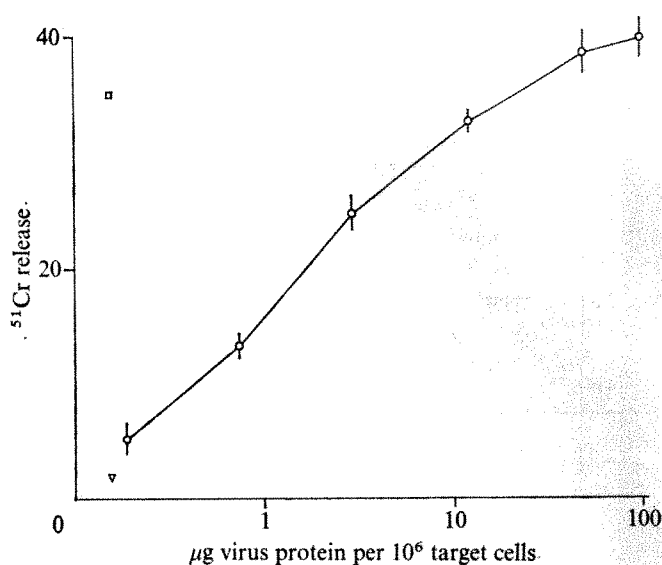


Fig. 2 T cell-mediated lysis of P-815 target cells exposed to dilutions of purified Sendai virus inactivated with β -propiolactone (B-PL). Sendai virus was grown in the allantoic cavity of 10-d-old embryonated fowl eggs and purified by centrifugation through a sucrose gradient (20–55% in PBS-A). The virus-containing band was collected and concentrated by centrifugation at 120,000g for 1 h. Inactivation was achieved by incubating the purified virus (2 mg per ml, 4×10^4 HAU per ml, $1 \mu\text{g} \approx 10^{11}$ virus particles) for 18 h at 4 °C with β -propiolactone (Sigma) at a concentration of 0.025%. β -propiolactone was inactivated by incubation for 30 min at 37 °C. Inactivation was tested by egg infectivity titration¹⁵. ○—○, Lysis of P-815 cells exposed 2 h before test with dilutions of B-PL-Sendai virus (100 μg virus per 10^6 cells, about 10^6 particles per cell). □, P-815 targets infected overnight with 20 PFU per cell (1 PFU ≤ 10 particles). ▽, Non-infected P-815 control cells. Assay conditions identical to Table 1, incubation time 3 h, ratio of killer-target cells, 5:1.

expression of viral proteins on the cell surface. The ability of Sendai virus to by-pass these processes may be due to its unique capacity to mediate cell-cell fusion, which could allow the insertion of large amounts of virus glycoprotein into the target cell membrane. From the dose-response curve (Fig. 2) of target cell formation with dilutions of β -propiolactone inactivated Sendai virus, it can be seen that about 10^6 particles per target cell were required to induce

Table 1 Lysis of syngeneic target cells incubated with infective and inactivated Sendai virus or vesicles containing viral envelope proteins

Responder cells	Stimulator cells	Culture day	Ratio K:T	Targets			
				P-815	P-815/Sendai	P-815/B-PL-S	P-815/Sendai env.
BALB/c, Sendai immune	BALB/c con A treated blasts, Sendai infected	5	10:1	1.1	43.5	42.0	18.1
			5:1	0.3	26.6	26.3	9.7
			2.5:1	1.7	13.9	15.1	6.3
BALB/c, Sendai immune	BALB/c con A treated blasts	5	10:1	2.8	0.5	1.3	0.4
			5:1	3.5	0.9	0.9	1.0
BALB/c, Normal	BALB/c con A treated blasts, Sendai infected	5	10:1	0.1	0.9	4.0	1.6
			5:1	0.6	0.4	3.6	0.5

BALB/c mice were immunised to Sendai virus by i.p. injection of 0.2 ml allantoic fluid containing 10^7 plaque forming units (PFU) of virus³. Six weeks later spleens were collected and incubated in RPMI medium containing 10% foetal calf serum, glutamine and 5×10^{-6} M 2-mercaptoethanol in 2 ml well linbro plates in presence of stimulated cells. Stimulator cells were prepared by incubation of spleen cells in culture medium containing $2.5 \mu\text{g ml}^{-1}$ concanavalin A (con A) for 48 h followed by overnight infection with 20 PFU per cell Sendai virus and 2,000 rad irradiation. 5×10^6 responder cells were incubated with 5×10^5 stimulator cells. Cytotoxic T cells were collected from cultures 4–7 d later⁴ and a ^{51}Cr release assay was performed with 5×10^3 syngeneic tumour target cells per well⁵. Target cells were a, ^{51}Cr labelled non-infected P-815 cells; b, P-815 cells infected overnight with 20 PFU per cell Sendai virus; c, P-815 cells pre-incubated for 2 h with β -propiolactone (B-PL) inactivated (see legend Fig. 2), purified Sendai virus in a protein concentration of $50 \mu\text{g}$ per 10^6 target cells; d, P-815 cells pre-incubated for 2 h with Sendai envelope (env.) proteins (see legend to Fig. 1) in a protein concentration of $50 \mu\text{g}$ per 10^6 target cells. Results are from triplicate assays; S.E.M. were always less than 3. Spontaneous lysis, which did not exceed 15–20%, is subtracted.

Table 2 Lysis of target cells treated with β -propiolactone inactivated Sendai virus is *H*-2 restricted

Responder cells	Stimulator cells	P-815 S-B-PL (<i>H</i> -2 ^{dd})	L-929 S-B-PL (<i>H</i> -2 ^{kk})	EL-4 S-B-PL (<i>H</i> -2 ^{bb})
BALB/c normal (<i>H</i> -2 ^{dd})	BALB/c con A treated blast, Sendai infected	3.1	1.4	2.5
BALB/c immune (<i>H</i> -2 ^{dd})	BALB/c con A treated blasts, Sendai infected	46.1	1.5	11.9
CBA immune (<i>H</i> -2 ^{kk})	CBA con A treated blasts, Sendai infected	2.4	30.6	3.0
C57BL6 immune (<i>H</i> -2 ^{bb})	C57 BL6 con A treated blast, Sendai infected	—	3.8	32.5

Conditions of immunisation and *in vitro* restimulation were as for Table 1. Cells were collected and tested on culture day 6. Target cells were incubated with B-PL-virus, in a protein concentration of 50 μ g per 10⁶ target cells. Results are from triplicate assays, ratio of killer-target cells is 20:1, incubation time 4 h.

a comparable degree of killing as seen with infected target cells. Since the percentage of particles which actually fuse with the target cells is unknown, the number of fused particles per cell necessary for target cell formation cannot be estimated. Failure to use sufficiently high concentrations of virus during target cell incubation may explain previous negative findings (ref. 12, and U.K., unpublished).

β -propiolactone-Sendai virus-treated target cells were lysed to a similar extent as target cells incubated with live virus, but target cells which were produced with Sendai virus envelope vesicles were consistently lysed at a lower plateau level. Two explanations are possible. There could be two populations of Sendai virus specific cytotoxic T cells

within'. If there are alterations of *H*-2 gene products these must occur from without, at the cell surface. Envelopes contain neuraminidase activity, but cleavage of neuraminic acid seems not to change the *H*-2 surface antigen specificity^{16,17}. We do not know if there are other residual enzyme activities such as proteases or glycosidases remaining in the envelopes. It is also possible that cross-linking of sialic acids by the viral haemagglutinin might change the surface distribution of *H*-2, and sometimes create effective target antigens.

Which then are the viral antigens on cell surfaces recognised by the T cells? Recent studies on influenza virus-specific CTL have suggested that the M-protein which is

Table 3 Virus-specific lysis of target cells incubated with inactivated Sendai virus or Sendai virus envelopes

Responder cells	Stimulator cells	Culture day	Ratio K:T	Targets				
				P-815	P-815/influenza	P815/Sendai	P-815/B-PL-S	P-815/Sendai env.
BALB/c, Sendai immune	P-815, Sendai infected	5	10:1	4.3	2.3	39.6	28.1	19.3
			5:1	-2.5	3.0	37.8	19.8	14.2
BALB/c, influenza immune	P-815, influenza infected	5	10:1	-0.9	27.4	0.8	1.3	1.8
			5:1	0.2	13.9	0.3	-3.8	3.9
(C3H $\times$ DBA/2) F ₁ , Sendai immune	BALB/c LPS blasts, Sendai infected	5	10:1	-0.29	3.7	26.5	27.2	—
			5:1	1.02	1.4	8.6	8.5	—
(C3H $\times$ DBA/2) F ₁ , influenza immune	BALB/c LPS blasts, influenza infected	5	10:1	0.3	19.8	3.4	3.8	—
			5:1	2.9	11.3	0.5	2.9	—

Responder cells (BALB/c, (C3H $\times$ DBA/2) F₁) were collected from mice injected i.p. 6 weeks previously with 10⁷ PFU Sendai virus or influenza virus. The influenza strain A/Jap/Bel/305/57 (H₂N₂) a recombinant of A/Jap/305/57 (H₂N₂) and A/Bel/42 (H₀N₁) (kindly supplied by Dr J. Skehel) was used. Conditions of *in vivo* sensitisation and *in vitro* boosting of cells were similar to those used for secondary cytotoxic T-cell generation against Sendai virus. Stimulator cells were BALB/c lymphocyte blasts after 48 h incubation in 50 μ g ml⁻¹ lipopolysaccharide containing medium or P-815 tumour cells. Stimulator cells were infected overnight with 20 PFU per cell and irradiated with 2,000 rad (blasts) or 8,000 rad (P-815). Ratio of stimulator to responder cells and ⁵¹Cr release assay conditions were identical to those described in the legend to Table 1.

with receptors for different viral antigens; however, antigens on envelope-treated target cells are at least partially shared with those of infected target cells since the latter compete in cold cell inhibition tests with the lysis of target cells exposed to Sendai virus envelopes (data not shown). The second possibility is that the envelope vesicles are less active in fusing with the target cell membrane; this is the more likely explanation since the β -propiolactone inactivated virus was active in cell-cell fusion, while the envelopes were not.

These results rule out, at least for Sendai virus infection, formation of an 'altered self' by a 'modification from

not glycosylated and not expressed on cell membranes may contribute to target specificity¹⁸. This is very unlikely for Sendai virus since the envelope preparations contain only two glycoproteins, one with neuraminidase and haemagglutinin activity and the other with fusion activity.

These results show for the first time that target cells for virus-specific T-cell lysis can be prepared with vesicles containing essentially only viral surface glycoproteins and that viral protein synthesis is not required for target cell formation.

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Cellular inactivation by ultrasound

THE lethal effect of ultrasound (US) on mammalian cells has received relatively little attention. Understandably, potential genetic aspects of US have been of prime concern to physicians who use US as a diagnostic tool; at the average power densities involved ($\ll 1 \text{ W cm}^{-2}$) little, if any cell killing is to be expected. There have been sporadic attempts to use higher intensities ($\sim 1 \text{ W cm}^{-2}$) as a treatment modality in cancer therapy^{1,2}, but those experiments seem to have been based on inadequate cellular studies. The effects of US usually were evaluated in terms of morphological criteria rather than on quantitative determination of the loss of viability as measured by colony formation. There are few reports of the effects of US on survival of mammalian cells^{3,4}, and none specifically examine hyperthermic interaction. With the increased interest in hyperthermia for tumour therapy, attention has been directed towards the use of ultrasound to achieve tumour heating. In preliminary experiments in which US was used to heat the EMT6 sarcoma and KHJ carcinoma in mice, we found a high percentage of tumour cures with short (~ 30 min) treatments at temperatures ($43-44^\circ\text{C}$) where *in vitro* results of hyperthermia-induced cell killing⁵⁻⁷ would not have led to a prediction of any cures. We therefore initiated an investigation of the effects of US on survival of Chinese hamster cells to see if direct cell killing by US could explain our *in vivo* results, or, as in the case of radiofrequency (RF) electromagnetic heating, we would be forced to invoke host response⁸. In particular, we examined the thermal and non-thermal components of cellular inactivation by US. We report here that there is a definite non-thermal cytotoxic effect of US. Its relative contribution to cell killing is a highly nonlinear function of the temperature of the cellular milieu. The survival curves show clearly that, beyond an initial threshold, small changes in temperature and/or US intensity can give rise to impressive changes in survival values. The threshold nature of the data strongly suggests that by means of overlapping beams, ultrasound energy could be delivered to tumour tissue to achieve massive cell killings while

sparing normal tissue outside the tumour volume to a degree far exceeding that of conventional techniques.

Chinese hamster cells (HA-1) of ovarian origin were maintained in Eagle's MEM supplemented with 15% foetal calf serum, penicillin, and streptomycin. The cultures were kept in a humidified incubator with a mixture of 95% air and 5% CO_2 , and were routinely checked for mycoplasma. To maximise the transmission of the acoustic energy and hence minimise heat induced by absorption of US, cells were grown as monolayers in specially designed stainless steel dishes with Mylar bottoms ($130 \mu\text{m}$ thick). Approximately 8×10^4 cells were seeded within a confined area (1.6 cm in diameter) on the thin Mylar film; experiments were always performed on the third day after plating when the cell density reached approximately $5 \times 10^5 \text{ cells cm}^{-2}$ and the cells were still in exponential growth. Plating efficiencies of cells trypsinised from the Mylar film were 70-90%. Some experiments were performed with cells in suspension; as far as possible, the geometry was identical to that used for attached cells. The cloning technique of Puck and Marcus was used to determine the fraction of viable cells. The US irradiating system consisted of a laboratory-developed signal generator and power amplifier driving a piezo-electric transducer (Mettler Electronics). The transducer was mounted on the frame

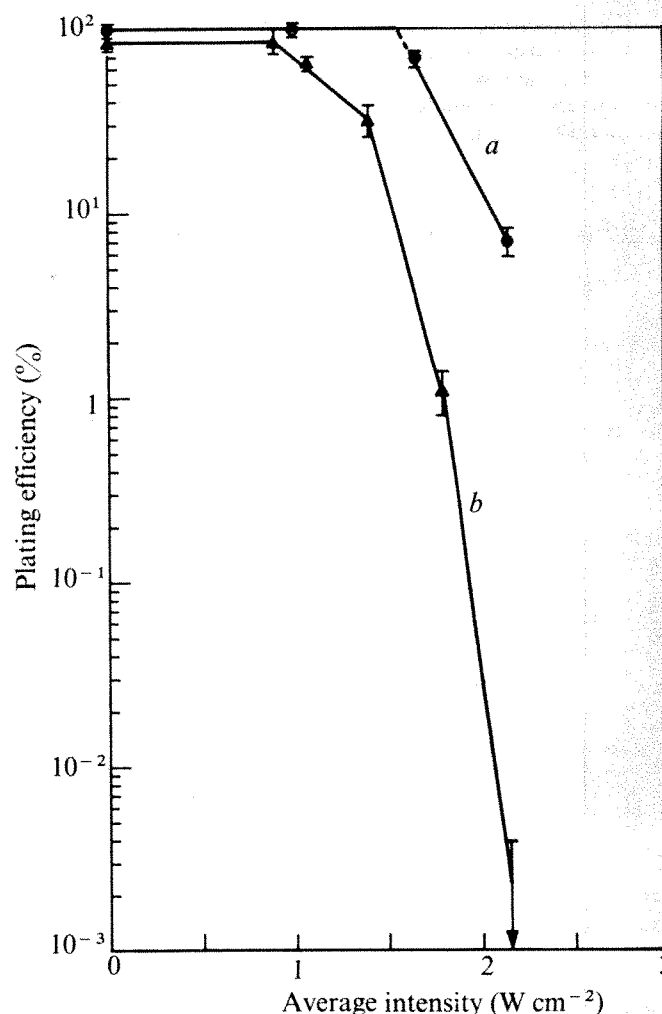


Fig. 1 Plating efficiencies (normalised to untreated controls) against US intensity. Cells were irradiated for 30 min at a, 37°C ; b, 43°C . Error bars in this and the succeeding figures show s.e.m. Plating efficiency is defined as (no. colonies per dish/no. cells plated per dish) $\times 100$. Cells plated per dish represents countable cells obtained by enzymic removal from Mylar after treatment.

of a specially designed dish holder, in a circulating hot water bath (Forma-Temp. Jr), so that alignment between cells and the transducer was accurately reproducible. The Mylar bottom of the dish was always in direct contact with circulating water of the desired temperature. The cells were irradiated at a distance of 13 cm from the transducer (3.5 cm diameter). The operating frequency was 1 MHz and the signal was pulse-modulated at about 1,000 cycles s^{-1} (duty cycle 60%). The irradiation field was 1.6 cm in diameter at this source-target distance; the spatial uniformity of the field intensity was within $\pm 20\%$ as measured by an absorbing probe. This probe consisted of a 22 gauge needle with a thermistor mounted inside the top; the tip was surrounded by a cylinder of silicon rubber (3 mm in diameter and 5 mm in length). The probe was calibrated by RCA laboratories, Princeton, New Jersey; the intensity measured included any reflected contribution. Agitation minimised any standing wave formation, however. The temperature of the water bath was maintained to $\pm 0.1^\circ C$; the temperature difference between the circulating water and the medium inside the dish was less than $0.1^\circ C$ even at US intensities of $2 W cm^{-2}$. The time required to reach thermal equilibrium was about 2–3 min; this is included in the quoted times of heating and/or US irradiation. Temperature measurements in the presence of US fields were obtained using thermocouples encased in 28 gauge needles. At intensities of approximately $0.7 W cm^{-2}$ at the measuring instrument, we saw no interaction of US and the sensor. When higher intensities were used, such interaction was observed and had to be taken into account; an infrared pyrometer was then used to measure the temperature of the medium inside the dish.

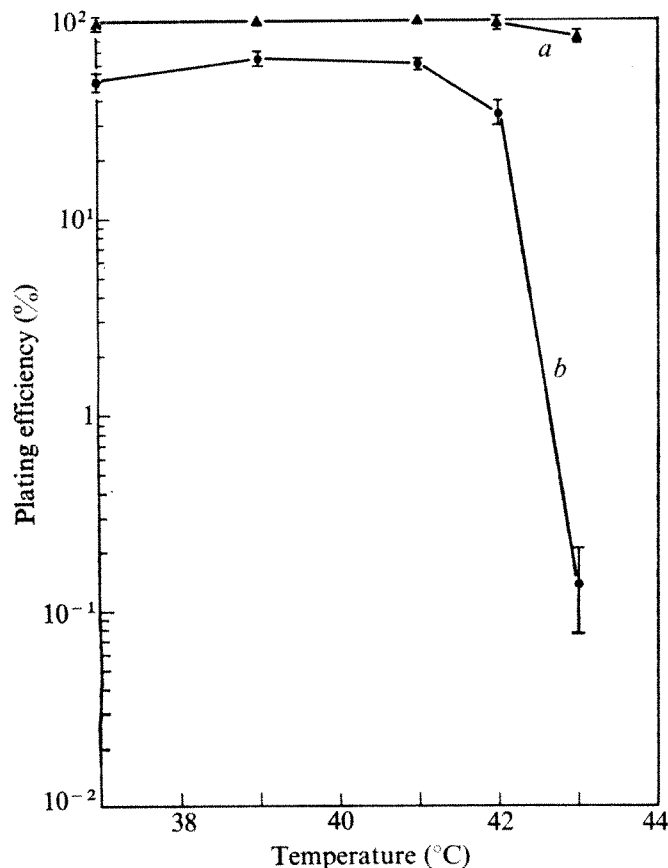


Fig. 2 Plating efficiencies (normalised to untreated controls) against temperature. *a*, Cells exposed for 30 min to heat alone; *b*, cells irradiated for 30 min with US ($2 W cm^{-2}$) at the indicated temperatures.

For each experiment, survival values of cells exposed to heat treatment in the absence of US irradiation were measured; the results were all in good agreement with previously published data⁶ and were used as thermal controls. Any cytotoxicity above these control values is attributed to non-thermal mechanisms.

Monolayers of cells were exposed to pulsed US radiation for 30 min at various average intensities and at water bath temperatures ranging from 37 to 45 °C. Plating efficiencies (all normalised to those of untreated controls) of cells irradiated at 37 °C and 43 °C are shown in Fig. 1. The cytotoxicity of US irradiation had a threshold when plotted against average acoustic intensity. This threshold intensity was temperature dependent: at 43 °C it was lower than

Table 1 Effect of ultrasound radiation on cell number

Average intensity ($W cm^{-2}$)	Countable cells on Mylar after 30 min US irradiation (% of unirradiated controls)	
	37 °C	43 °C
0.75	100	100
1.5	18	20
2.0	5	5

37 °C. Cells were then exposed at various temperatures either with or without pulsed ultrasound at an average intensity of $2 W cm^{-2}$. Figure 2 shows the plating efficiencies as a function of water bath temperature. Survival of cells exposed simultaneously to heat and ultrasound was lower than that of cells exposed to heat alone. Plotted against temperature, the cytotoxicity of US irradiation at a constant average intensity again showed a threshold.

In addition to changes in plating efficiency, there was also a reduction in cell number, showing the occurrence of immediate cell lysis. Table 1 indicates the percentage of cells which were attached to the Mylar after 30 min exposure to US, with respect to the untreated control. The cell number includes both viable and non-viable cells. In contrast to plating efficiency, the fraction of cells lysed showed no significant temperature dependence between 37 and 43 °C. Survival values, after correction for cell lysis, are shown in Fig. 3 for one set of experiments. The number of cells recovered from the supernatant was negligible.

The possibility that these results were artefact of the attachment of cells to the Mylar was ruled out by the finding that cells in suspension responded similarly to US irradiation (data not shown). The nature of US-induced cell damage is not clearly understood. In general, US effects are classified under thermal, cavitation or 'other'⁹⁻¹¹. In the experiments described above, the magnitude of thermal effects has been evaluated independently, since the macroscopic temperature in each case was established by the hot water bath and not by the US intensity. The cell killing over and above that caused by this temperature is clearly due to non-thermal effects. These non-thermal effects may be divided into temperature-dependent and temperature-independent components. Cell lysis is largely temperature-independent, while cell inactivation, as measured by the reduction in plating efficiency, results at least in part from temperature-dependent effects. Cavitation played an important part; this was shown in experiments in which the average US energy was kept constant, but pulse duration and repetition frequency were varied. With pulses of less than 1 ms, much less cell lysis and cell death were seen; 1 ms is about the minimum time required for the onset of cavitation¹². Even much shorter pulses induced considerable lysis and cell death, however; hence other phenomena such as, for example, acoustic streaming were involved.

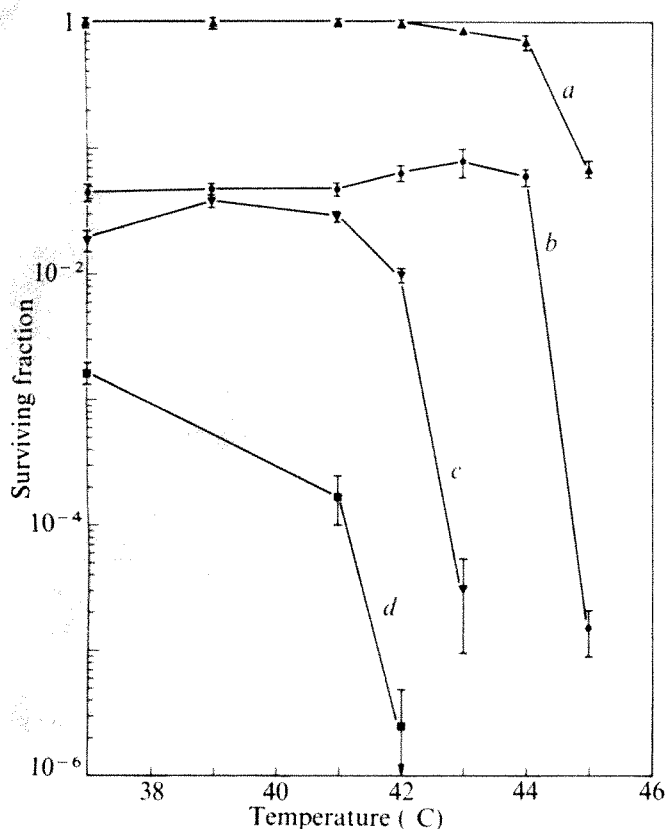


Fig. 3 Surviving fractions of cells exposed to US for 30 min at various temperatures and intensities. *a*, Heat alone; *b*, 1 W cm⁻²; *c*, 2 W cm⁻²; *d*, 2.2 W cm⁻². Surviving fraction (SF) is defined as (cell no. after treatment/cell no. before treatment) × (plating efficiency).

The dramatic temperature-dependent changes in plating efficiencies observed (Figs 1 and 2) are unlikely to be related to changes in the physical action of ultrasound, but rather to intracellular changes. Experiments in ours and other laboratories have demonstrated a variety of temperature-related changes in the response of cells to cytotoxic agents¹³, to hypo- or hypertonic environments¹⁴, and to membrane-specific antibiotic amphotericin B (ref. 14). These changes, which probably involve modification of cellular membranes, occur in the range of 41–43 °C, and all are in the direction of appreciably and sometimes dramatically greater sensitivity to the external challenge. Thus, it is not unreasonable to find that the cells' response to US also changes drastically with temperature. It must be kept in mind, however, that the observations of thermal interactions depend critically on the particular parameters of US irradiation. Intensity variations, frequency variations (or modulation) and changes in pulse width can shift the thresholds appreciably. This makes it imperative that, for comparison of results, conditions be standardised, and that the details of US irradiations be reported carefully.

The shapes of the survival curves (Figs 1–3) suggest that US may be a very useful tool in the eradication of localised tumours. Since US can readily be focused, it should not be difficult to obtain 'dose distribution' such that temperature and intensity thresholds are exceeded only within a hypothetical volume. Within that volume, extensive cell killing would then be effected, while in the surrounding normal tissue very little cytotoxicity would be expected. In that sense, then, US would differ greatly from X-ray (or even high LET particle) therapy, as well as from focal ultrasound ablation¹⁵, where normal tissue toxicity outside the tumour volume is frequently the treatment-limiting consideration. It would also differ from RF-induced current

heating, where cell inactivation seems to be largely (or exclusively) through thermal mechanisms⁸. The tumour experiments referred to earlier are consistent with this expectation: in mice which had been cured of their tumours by US and which were sacrificed as late as 180 d after treatment, little or no evidence of normal tissue damage was seen (Fajardo, Marmor and Hahn, unpublished). We are not sure, however, that the tumour cures can be explained entirely on the basis of this direct US-related cell killing: at the intensity levels of the tumour irradiations, at least the peripheries of the tumours should have been below the cytotoxic threshold levels found *in vitro*.

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Effect of human interferon preparations on lymphoblastogenesis in Down's syndrome

THE best characterised properties of human interferon, its antiviral (AV) and cell multiplication inhibitory (CMI) activities, are controlled, in an unexplained manner, by genes on chromosomes 21 (refs 1–4; 14–16). Human and animal interferons have various immunosuppressive effects^{3–10}, among them the inhibition *in vitro* of DNA synthesis in activated lymphocytes¹¹. Using mitogen- and antigen-stimulated lymphocytes from normal subjects (disomic 21) and others with Down's syndrome (trisomic 21), we have found that DNA synthesis is inhibited to a greater degree in the latter by both fibroblastoid and leukocyte interferons. We suggest that this property is also regulated by genes on chromosome 21.

Blood samples were obtained from ten subjects previously diagnosed with Down's syndrome, and from seven normal volunteers. The former were karyotyped, using quinacrine staining, to verify trisomy of chromosome 21. Lymphocytes were separated on a Ficoll-Hypaque gradient. Blast formation was stimulated either with phytohaemagglutinin (PHA) or in a one-way mixed lymphocyte reaction (MLR). Serial dilutions of two different interferon preparations, one from a poly(I)-poly(C)-induced fibroblastoid cell line maintained in our laboratory³ and one from virus-induced leukocytes, were added at the beginning of culture. Serially diluted

Table 1 Sensitivity of trisomic 21 lymphocytes to interferon preparations

Trisomic 21 donor	Age	Fibroblast interferon		Leukocyte interferon	
		PHA	MLR	PHA	MLR
M.B.	16	4	1.5	4	1
N.C.	17	2	3	4	1.5
H.C.	28	25	32	1,000	—
J.S.	28	1	1	—	—
R.D.	32	2	4	850	6
D.B.	32	2	7	850	2
L.G.	9	11	—	30	4
S.N.	8	18	—	70	2
R.H.	14	5	5	85	7
D.L.	13	3	5	30	5

dilution of interferon preparation that produces 50% inhibition of DNA synthesis in trisomic 21 lymphocytes

Sensitivity = $\frac{\text{dilution of interferon preparation that produces 50\% inhibition of DNA synthesis in trisomic 21 lymphocytes}}{\text{dilution of interferon preparation that produces 50\% inhibition of DNA synthesis in disomic 21 lymphocytes}}$

Two trisomic 21 and one disomic 21 samples were tested on each occasion. Human fibroblast interferon was prepared by inducing human fibroblastoid cultures with poly (I)·poly (C). This interferon preparation was centrifuged at 100,000g for 1 h to remove any SV40 virus particles. Purified human leukocyte interferon¹³, prepared by Dr K. Cantell, was supplied by Dr M. Krim. Both interferon preparations were verified by Dr E. Havell as fibroblast and leukocyte interferon, respectively, by antibody neutralisation tests¹². Lymphocytes were cultured and stimulated with PHA or by allogeneic cells as described in Tables 2 and 3, and were treated with serially diluted interferon preparations for the duration of the experiments. Mock interferon preparations of the appropriate dilution were included with the experiments.

mock interferon, prepared in the same way as fibroblastoid interferon, but without inducer, was added to control cultures. DNA synthesis, an indicator of lymphoblastogenesis, was determined by measuring the incorporation of tritiated thymidine into the trichloroacetic acid (TCA)-precipitable cell fraction. To ensure that the interferon preparations did not alter the uptake of thymidine into the

Table 2 DNA synthesis in PHA-stimulated lymphocytes and unstimulated controls

Expt no.	Disomic 21		Trisomic 21	
	PHA	Control	PHA	Control
(1)	20,369	94	13,638 24,284	197 114
(2)	32,770	—	26,997 20,570	—
(3)	23,241	491	21,176 14,110	410 203
(4)	26,154	—	15,186 12,680	—
(5)	35,431	437	35,973 27,314	185 286

Figures are average c.p.m. from duplicate cultures.

Lymphocytes were separated from heparinised blood on a Ficoll-Hypaque gradient (Pharmacia) and washed three times in Ca^{2+} and Mg^{2+} free Hanks basic salt solution (Gibco). Duplicate sets of cultures were grown in round bottomed Microtest plates (Falcon) using RPMI 1640 medium (Gibco) containing 10% foetal calf serum (Flow), 1% chlortetracycline and 0.1% PHA-P (Difco). Each well contained 5×10^4 cells in 0.2 ml. After 72 h, 0.01 ml of ^3H -thymidine (Schwartz-Mann, specific activity 1.9 Ci mmol⁻¹, 0.1 mCi ml⁻¹) was added. Four hours later, 20 μl of 20% sodium dodecyl sulphate was added to lyse the cells. The contents of each well were placed on glass fibre filter papers, washed three times in 5% TCA to precipitate the DNA and rinsed three times with 95% ethanol. After drying, the papers were placed in liquid scintillation vials with Spectrafluor (6 g PPO and 75 mg POPPOP per l toluene, Amersham/Searle) and counted for 10 min, or to 1% error.

cell, the amount of radioactivity in the soluble fractions was also measured; no difference was found between the interferon-treated and untreated cultures.

In similar experiments, human interferon preparations were applied to PHA-stimulated murine spleen cells, while mouse interferon was added to human lymphocyte cultures. No species cross-reactivity was observed.

Trisomic 21 lymphocytes, whether stimulated by PHA or by allogeneic cells, were more sensitive than disomic 21 lymphocytes to the suppression of DNA synthesis by both interferon preparations (Table 1). The number of copies of chromosome 21 had no effect on the maximum levels of DNA synthesis obtained in the mock interferon-treated controls (Table 2). There was also no significant difference ($P > 0.5$) in maximum levels of DNA synthesis between the various donor combinations in the MLR, that is, it made no difference which genotype, trisomic 21 or disomic 21, was the stimulator or responder (Table 3). These results

Table 3 DNA synthesis in one-way mixed lymphocyte reactions

Expt no.	Responder $\times$ stimulator		T21 $\times$ T21
	T21 $\times$ D21	D21 $\times$ T21	
(1)	4,053 2,659	2,858 4,402	4,227 4,865
(2)	2,217 2,679	4,244 5,621	2,667 2,295
(3)	7,156 7,148	8,161 6,625	5,484 9,044
(4)	6,428 3,212	5,151 7,539	3,853 1,914
(5)	4,208 3,237	5,668 4,052	1,638 1,850

Figures are average c.p.m. from duplicate cultures. T21, trisomic 21; D21, disomic 21.

After separation, lymphocytes to be used as stimulator cells were treated for 30 min with mitomycin C ($25 \mu\text{g ml}^{-1}$), and washed twice in RPMI 1640 medium. Duplicate sets of cultures consisted of 5×10^4 stimulator and 5×10^4 responder cells in each well with 0.2 ml of complete medium. ^3H -thymidine (0.01 ml) was added after 96 h and cultures were terminated at 120 h.

indicate that the higher sensitivity of trisomic 21 lymphocytes to human interferon preparations is due to the presence of an extra copy of chromosome 21, and not to a changed sensitivity to antigenic or mitogenic stimuli.

The lymphocytes of one individual, J.S., who had been suspected to have Down's syndrome, showed a response to interferon similar to those of disomic 21 controls (Table 1); karyotyping revealed that J.S. had only two copies of chromosome 21. All trisomic 21 lymphocytes were more sensitive to the inhibitory effects of the human interferon preparations. This suggests that suppression of *in vitro* lymphoblastogenesis by human interferon, like its AV and CMI activities, acts through genes on chromosome 21. This implies that the various actions of human interferon applied to human cells are mediated through a common cellular pathway, the exact nature of which will probably not be known until the specific gene product(s) of chromosome 21 has been identified. Because interferons have not been purified and isolated conclusively, it remains to be seen whether the different effects of interferon preparations are due to one or more molecules. Until purification is achieved, and the respective antibodies prepared, it should be possible to assay human interferon preparations on the basis of their action through chromosome 21, in order to distinguish them from other macromolecules with similar actions, such as other lymphokines.

The developmental and metabolic abnormalities associated with Down's syndrome are probably due to the

presence of an extra chromosome and the resulting imbalance of gene products. Since trisomic 21 cells are hypersensitive to the CMI activities of human interferons, including the inhibition of lymphoblastogenesis, it is possible that some of the developmental and immunological defects associated with Down's syndrome are due to an abnormal interferon response.

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Dystrophic mice show age related muscle fibre and myelinated axon losses

ALTHOUGH many differences between age matched normal and dystrophic animals have been found, there have been few demonstrations of a time related quantitative change from normal to a characteristically dystrophic situation within the dystrophic strain itself. Here we report such a change—normal numbers of muscle fibres present in young dystrophic mice were rapidly lost, such that older animals showed the reduced number of muscle fibres characteristic of murine dystrophy. Although these losses began after a demonstrable loss of myelinated axons had occurred, it is not possible to say if the loss of muscle fibres was a result of the loss of nerve fibres.

The major practical problem in this study was that of obtaining animals of known dystrophic genotype of less than 3-4 weeks of age. The use of the C57BL/6J dy^{2J}/dy^{2J} strain of dystrophic¹ mice overcame this problem; affected animals occasionally breed and produce one or two litters. Thus, with animals of known dystrophic genotype, early developmental observations can be carried out.

A detailed investigation of the growth and development of normal 129ReJ $+/+$ and dystrophic 129ReJ dy/dy mouse muscles^{2,3} revealed a progressive loss of muscle fibres from the earliest age point at about 3 weeks. By about 6 weeks 40-50% of the muscle fibres, when compared with normal, had been lost from the respectively fast⁴ and slow-twitch⁴ extensor digitorum longus (EDL) and soleus muscles. Harris *et al.*⁵ and Bradley and Jenkinson⁶ have published conflicting reports on the number of myelinated axons in the nerves of 129ReJ dy/dy dystrophic mice. A 20% reduction of myelinated axons was reported by Law and Atwood⁷ in 129ReJ dy/dy dystrophic mouse soleus nerves. Bray and Aguayo⁸ observed a slight but probably not statistically significant reduction in the number of

myelinated axons in the nerve to the medial gastrocnemius muscle of 129B6F₁ dy/dy dystrophic mice.

Each of the studies mentioned above used a light microscope counting technique for axon numbers. We have found that these techniques often lead to an underestimation by 10-20% of the number of myelinated axons present when compared with counts obtained from low-power electron micrographs of the same nerve. We therefore used low-power electron micrographs of the nerves of male and female C57BL/6J $+/+$ normal and C57BL/6J dy^{2J}/dy^{2J}

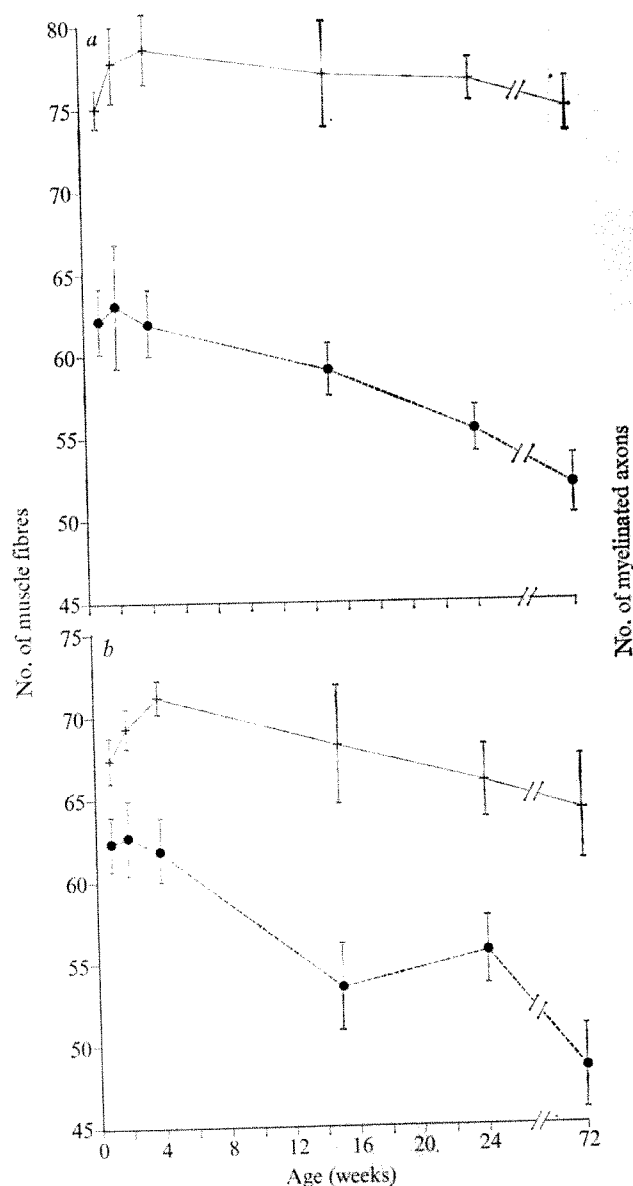


Fig. 1 *a* Muscle fibre counts from normal (+) and dystrophic (●) soleus muscles as a function of age. Each point represents the mean $\pm$ s.e. of at least 6 observations. The 1 week normal and dystrophic values were not statistically significantly different (Student's *t* test $P=0.05$ was used for all significance tests). At 15 and 72 weeks the percentage reductions in dystrophic fibre numbers were 37.4% and 33.8% respectively and the differences between the dystrophic and normal mean values at these age points were significantly different. *b*, Muscle fibre counts from normal (+) and dystrophic (●) extensor digitorum longus muscles. Each point represents the mean $\pm$ s.e. of at least 6 observations. The differences between the 1, 2 and 4 week normal and dystrophic values were not statistically significant. At 15 and 72 weeks the percentage reductions in dystrophic fibre numbers were 45.9% and 44.6% and the differences between the dystrophic and normal mean values at these age points were significantly different.

dystrophic animals aged 1–72 weeks. The nerves used in this study were those innervating the fast-twitch plantaris⁹ and slow-twitch⁴ soleus muscles. They both presented easily accessible, anatomically distinct, unifascicular portions,

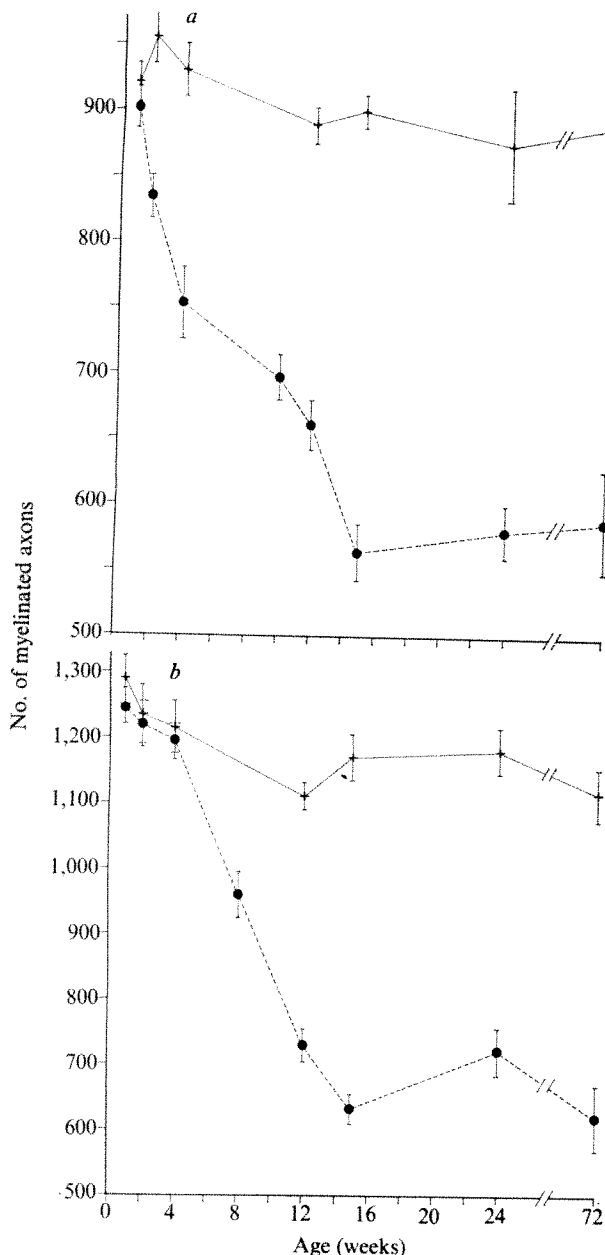


Fig. 2 *a* Myelinated axon counts from normal (+) and dystrophic (●) soleus nerves as a function of age. Each point represents the mean $\pm$ s.e. of at least 6 observations. The regression line for normal nerves was $y = -0.035x + 77.7$ and for dystrophic $y = -0.152x + 61.9$. The regression coefficient for normal nerves was not significantly different from zero whereas that for dystrophic nerves was. At 1 week the percentage reduction of dystrophic myelinated axons was 18.0% and at 72 weeks 30.8% and the difference between the normal and dystrophic mean values was statistically significant at every age point. The difference between the 1- and 72-week dystrophic values was significant and the percentage reduction in this case was 16.3%. *b*, Myelinated axon counts from normal (+) and dystrophic (●) plantaris nerves. Each point represents the mean $\pm$ s.e. of at least 6 observations. The regression line for normal values was $y = 0.074x + 69.2$ and for dystrophic values $y = 0.189x + 61.0$. The regression coefficient for normal nerves was not significantly different from zero whereas that for dystrophic nerves was. At 1 week the percentage reduction of dystrophic myelinated axons was 7.6% and at 72 weeks 24.3%. The differences between normal and dystrophic 1- and 2-week mean values were not statistically significant, at every other age point the differences were significant. The difference between the 1-week and 72-week dystrophic values was significant and the percentage reduction in this case was 21.9%.

from which transverse sections could be cut. After fixation for 2 h at 4 °C with a 3% solution of glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.4, the nerves were removed and placed in a 2% osmium tetroxide solution in 0.1 M sodium cacodylate buffer pH 7.4 at 4 °C for 1 h. After dehydration and embedding in Epon-Araldite, thin sections were cut for viewing with a Philips 200 electron microscope. The pennate structure of the plantaris muscle made it unsuitable for obtaining a count of the total number of muscle fibres from one transverse section. The EDL muscle, which histological examination had shown to be affected by the disease process, was therefore used as a fast-twitch muscle from which to obtain muscle fibre numbers. The slow-twitch soleus muscle was also studied. Muscles were fixed at their *in vivo* lengths with formol saline (NaCl 0.9%, formaldehyde 4.0%) then removed and treated for 1 h with 10% sulphuric acid to partly separate the fascicles¹⁰. Paraffin wax sections of 7 μ m thick were then cut from the belly of the whole muscle and stained with haematoxylin and eosin. Counting was carried out with the aid of a Leitz Prado microscope slide projector.

Figure 1 shows that rapid early reductions in the numbers of muscle fibres occurred in both soleus and EDL muscles. The percentage reductions in the EDL muscles were somewhat larger than in the soleus muscles and also began a little later. These changes were similar in magnitude to those observed by Rowe and Goldspink³ but, whereas the reduction in the 129ReJ *dy/dy* dystrophic mouse muscles were completed by about 6 weeks of age, those in the C57BL/6J *dy^{2j}/dy^{2j}* dystrophic mice were not completed until about 15 weeks of age. This again raises the question of two allelic genes having different phenotypic expression¹¹. The myelinated axon counts are shown in Fig. 2. In both dystrophic soleus and plantaris nerves the number of myelinated axons was always less than in their normal counterparts at each of the age points studied; these differences were significant. The percentage reduction in the myelinated axons of dystrophic soleus nerves was greater at every age point than the percentage reduction in the dystrophic plantaris nerves. At no age point was the percentage reduction in the nerve to the fast-twitch dystrophic plantaris muscles as great as that reported for the nerve³ to the fast-twitch^{12,13} tibialis anterior muscles. A progressive reduction in the number of myelinated axons also took place in both dystrophic plantaris and soleus nerves such that the 1- and 72-week values for each nerve were statistically significantly different. In addition, the regression coefficients were statistically significantly different from zero while those of normal values were not.

The areas of large unmyelinated axons, previously described in the C57BL/6J *dy^{2j}/dy^{2j}* dystrophic mice¹⁴, were not seen in the distal nerves we studied. We are also not yet able to say whether the myelinated axons that were lost were motor or sensory or both.

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Cs⁺ causes a voltage-dependent block of inward K currents in resting skeletal muscle fibres

WHEN frog skeletal muscle fibres are bathed in solutions containing Cs⁺ and K⁺ in the ratio 1:4,000, a reduction is observed in the size of inward K currents through the resting membrane. This effect is enhanced by an increase in either hyperpolarisation or external Cs⁺ concentration. It can be predicted from these findings that regenerative changes in membrane potential should be obtainable in fibres, in the presence of Cs⁺, that are hyperpolarised by means of a current electrode. Such responses are described in the last part of this report. In squid axon and frog node^{1,2}, internal Cs⁺ produces a voltage-dependent block of the delayed, outward K currents, though the ratio of Cs⁺ to K⁺ required for this effect is far greater than that used in the experiments reported here. A closer parallel can be drawn between our findings and those recently reported on the inward K currents in the starfish egg cell³.

Sartorius muscles of *Rana temporaria* were equilibrated in isotonic K₂SO₄ Ringer, containing 40 mM K₂SO₄, 8 mM CaSO₄, 1.08 mM Na₂HPO₄, 0.43 mM NaH₂PO₄ and 113 mM sucrose, pH 7.2. Additions of Cs₂SO₄ and K₂SO₄ were made by isotonic replacement of sucrose. Because K⁺ was the only permanent ion in these solutions, the resting potential was identical with the K equilibrium potential, V_K .

Membrane currents were measured using a three-electrode voltage-clamp technique⁴. Because with this method, membrane current is recorded as the potential difference between two intracellular microelectrodes, membrane current is expressed in mV. The membrane potential was held at V_K in all experiments.

Some properties of the resting K conductance are illustrated in Figs 1 and 2. The top of Fig. 1 shows records from two fibres, one (Fig. 1A) in 40 mM K₂SO₄, the other (Fig. 1B) in 40 mM K₂SO₄ plus 1.25 mM Cs₂SO₄. In the absence of Cs⁺ (Fig. 1A), the response to a step-wise hyperpolarisation to a membrane potential of -75 mV (upper record, a) was an inward current which turned on instantaneously and then declined slowly to a steady-state value. When the hyperpolarisation was increased (b), both instantaneous and steady-state currents were larger. The slow decline in K current has been attributed to a depletion of K⁺ adjacent to the membrane⁵. This depletion occurs because about 80% of the resting K conductance is located in the membrane of the transverse tubular system of muscle. During a maintained hyperpolarisation, K⁺ flowing into the sarcoplasm across the tubular wall is replaced partly by other ions from the bulk external medium, resulting in a fall in the tubular K⁺ concentration and consequent shift in V_K (ref. 6).

In the presence of 1.25 mM Cs₂SO₄ (Fig. 1B), both instantaneous and steady-state currents were smaller than for similar hyperpolarising voltages in the absence of Cs⁺. In addition, the current obtained for a large hyperpolarisation to -106 mV (lower record, b) was smaller than that recorded at only -81 mV (a). The ability of Cs⁺ to interfere with the inward movement of K⁺ must increase with hyperpolarisation. It is not clear from these records in Cs⁺ whether the time-dependent decline in current arises from a K depletion, a time-dependent block by Cs⁺, or both.

Mean instantaneous current-voltage relations for a number of fibres in solutions containing 0.01–1.25 mM

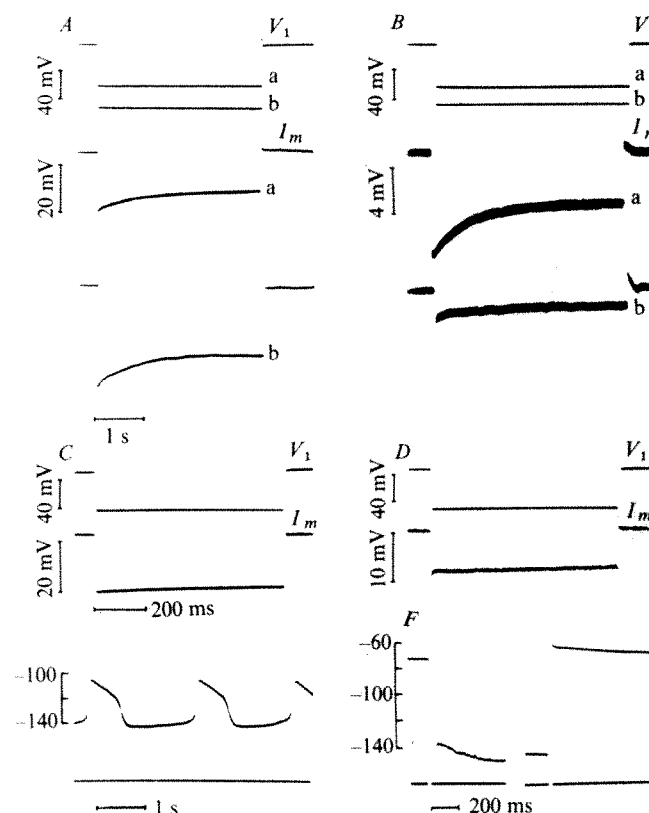


Fig. 1 A, records of membrane potential (above) and membrane current (below) from a fibre in 40 mM K₂SO₄ Ringer. Fibre resting potential (RP), -14 mV; holding potential (HP), -14 mV; T, 17.2 °C. B, as (A) except that the solution also contained 1.25 mM Cs₂SO₄. Fibre RP, -15 mV; HP, -15 mV; T, 17.2 °C. Time scale as in (A). C, record of membrane potential (above) and membrane current (below) from a fibre in 95 mM K₂SO₄ Ringer. RP, +3 mV; HP, +3 mV; T, 4 °C. D, as (C) except that the solution also contained 1.25 mM Cs₂SO₄. Fibre RP, +1 mV; HP, +1 mV; T, 3.8 °C. Time scale as in (C). E–F, records were made using the three-electrode voltage-clamp technique, where membrane current is recorded as the potential difference between two impaling microelectrodes, recording membrane potentials V_1 and V_2 , at distances l and $2l$ from the pelvic end of the fibre. The membrane current is then given by the approximation⁴

$$I_m = \frac{a(V_2 - V_1)}{3l^2 R_i} \text{ A cm}^{-2} \quad (1)$$

where a is the fibre radius and R_i is the resistivity of the sarcoplasm. The value of l was 330 μm. The calibration on the current records is in millivolts since the records are of $V_2 - V_1$. If a is assumed to be 40 μm, and R_i to be 180 Ω cm at 17–18 °C and 290 Ω cm at 3–4 °C, 1 mV equals 6.8 μA cm⁻² at 17–18 °C and 4.2 μA cm⁻² at 3–4 °C. E, record of membrane potential (above) and current flowing through an impaling microelectrode (below) made in constant current conditions with two impaling electrodes. The fibre is in a solution containing 40 mM K₂SO₄ and 0.25 mM Cs₂SO₄. Vertical scale: membrane potential (mV). F, records made as in (E), except that the fibre is in a solution containing 40 mM K₂SO₄ and 1.25 mM Cs₂SO₄.

Cs₂SO₄ are given in Fig. 2. In the absence of Cs⁺ (solid curve), the size of the K current depends on the direction in which K⁺ moves across the membrane. When the K-driving force (membrane potential, V_i , minus V_K) is negative, relatively large inward currents can be recorded. In contrast, when V_i minus V_K is positive and the K current is outward, currents are always small. The current-voltage relation is therefore that of an ingoing rectifier⁷.

Addition of Cs⁺ (Fig. 2) did not affect outward K currents. Similarly, when the inward driving force was small, inward currents also resembled those in the control Ringer. As V_i minus V_K was made more negative, however, the inward currents reached a maximum, then declined with further

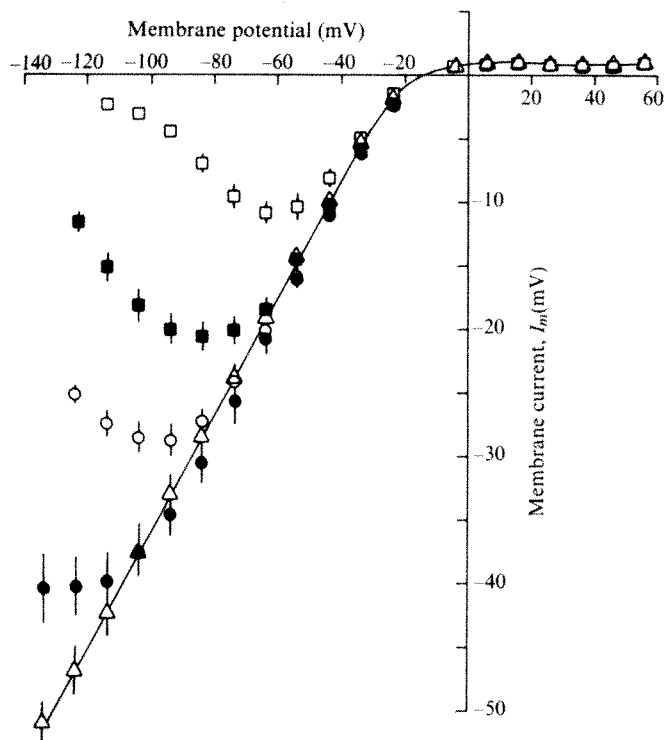


Fig. 2 Instantaneous current-voltage relations recorded with a three-electrode voltage clamp in muscle fibres. Abscissa, membrane potential, V_m (mV). Ordinate, membrane current, I_m (mV). In the solutions used the membrane current was carried by K^+ . The instantaneous current was obtained by exponential extrapolation of the time-dependent current (Fig. 1A and B) to the beginning of the voltage step. Each experimental point is the mean of results from six to eight fibres. Vertical lines, s.e.m. T , 17–18°C. Fibres were held at the resting potential (which was equal to V_K). RP, -14.13 ± 0.24 mV ($n = 48$) in 40 mM K_2SO_4 , and -13.92 ± 0.37 mV ($n = 24$) in 40 mM K_2SO_4 plus 1.25 mM Cs_2SO_4 (mean $\pm$ s.e.m. of all penetrations, n = no. of observations). Medium was 40 mM K_2SO_4 Ringer plus: Δ , no Cs_2SO_4 ; $\bullet$, 0.01 mM Cs_2SO_4 ; $\circ$, 0.05 mM Cs_2SO_4 ; $\blacksquare$, 0.25 mM Cs_2SO_4 ; $\square$, 1.25 mM Cs_2SO_4 .

hyperpolarisation. It can be concluded from these results that the block of instantaneous K currents by Cs^+ is a function of both the K-driving force and the external Cs^+ concentration.

To test whether the blockage by Cs^+ was purely instantaneous, currents were recorded in fibres in a 95 mM K_2SO_4 Ringer, in the cold. In these conditions the complications introduced by K depletion are much reduced, and the currents show very little time dependence in the control Ringer (Fig. 1C). Nor is there any noticeable time dependence (apart from the initial capacity transient) in the presence of 1.25 mM Cs_2SO_4 (Fig. 1D), which is consistent with the notion that the blockage is instantaneous, and has no time-dependent component.

The measurement of currents at extreme hyperpolarisation is complicated by contractile repriming and membrane breakdown. In solutions containing 40 mM K_2SO_4 plus 0.25 mM Cs_2SO_4 , however, instantaneous currents reached a minimum at -155 mV, then increased gradually with further hyperpolarisation. The current-voltage relation determined in the presence of Cs^+ for membrane potentials between -14 mV and -200 mV can therefore be intercepted at three membrane potentials by a line (drawn parallel to the voltage axis) corresponding to a small inward current. Because two of these potentials will be stable and one unstable, theoretically⁸ it should be possible to record regenerative responses from the unclamped, hyperpolarised membrane.

Muscle fibres were impaled near their mid-points by two electrodes, one to record the membrane potential, and the

other to pass current. When the hyperpolarisation produced by a steady inward current exceeded -115 mV in the presence of 0.25 mM Cs_2SO_4 , the membrane potential developed spontaneous, stable oscillations (Fig. 1E), with distinct thresholds for both a depolarisation and a repolarisation phase, reminiscent of the cardiac action potential. The depolarisation phase of the cycle is probably due to a regenerative increase in K permeability, produced by the potential-dependent dissociation of Cs^+ from the membrane. The potential slowly becomes more negative again as K depletion occurs, until a threshold is reached at which Cs^+ can again bind to the membrane, resulting in a regenerative decrease in K permeability and consequent rehyperpolarisation. That the thresholds and the peak depolarisation do not correspond correctly to predicted points on the current-voltage curve (Fig. 2) is in part due to the difference between the uniform and point methods of membrane polarisation⁸. In addition, the current-voltage relationship varies with time; this is also the reason why the responses are cyclical and not maintained.

Maintained, reversible transitions between two stable potentials (about -75 mV and -140 mV) were produced by brief current pulses superimposed on a steady inward current in 40 mM K_2SO_4 plus 1.25 mM Cs_2SO_4 (Fig. 1F). In these conditions, however, cyclical responses were never seen.

The ability to record two types of regenerative responses in hyperpolarised fibres also supports the conclusion that the blockage of inward currents by Cs^+ depends on both the membrane potential and the Cs^+ concentration. The stability of the responses indicates that the potential-dependent increase in instantaneous current at extreme hyperpolarisations is due to a recovery from Cs^+ blockage, and is not an artefact of membrane breakdown.

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Is hyperosmotic neurosecretion from motor nerve endings a calcium-dependent process?

SPONTANEOUS liberation of neurotransmitter quanta is strongly affected by the osmotic pressure of the extracellular fluid. Elevation of the osmolarity by 20–30% increases the rate of release from motor nerve endings by more than one order of magnitude^{1,2}. In this respect the neuromuscular junction resembles some other secretory systems^{3–5}. The mechanism of this hyperosmotic neurosecretion is not yet understood; extracellular calcium ions are not directly responsible, since this effect can be produced in their absence⁶. Recently, it has been suggested that the liberation of neurotransmitter is regulated by the intracellular concentration of free calcium ions^{7–8}. We have therefore examined the hypothesis that hyperosmotic neurosecretion originates from an increase in internal calcium concentration ($[Ca]_{in}$). At the frog neuromuscular synapse however, it is impossible at present to estimate directly free $[Ca]_{in}$; hence we used an indirect technique,

which is based on two assumptions: first, the frequency of the miniature endplate potentials (m.e.p.p.s.) reflects free $[Ca]_{in}$. Second, the movement of calcium ions across the presynaptic membrane is governed by the electrochemical gradient, and by the calcium conductance (g_{Ca}). If hyperosmotic neurosecretion is caused by an increase in $[Ca]_{in}$, then increasing g_{Ca} under reversed electrochemical gradient for the calcium should cause a reduction in the effect of hyperosmotic stress on transmitter release. We report that hyperosmotic neurosecretion is dependent on $[Ca]_{in}$.

The experiments were performed on the sartorius nerve muscle preparation of the frog *Rana pipiens*, using conventional methods for intracellular recording. The standard bathing Ringer solution contained 116 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$ (pH adjusted to 7.0 by titration). The extracellular calcium concentration was either 0.4 mM or less than 10^{-8} M when 1 mM EGTA was added⁹.

Figure 1 illustrates the main experimental observation. A preparation was bathed in a standard Ringer solution with 1 mM EGTA. At A, 50 mM sucrose were added to the medium. This hyperosmotic solution produced a marked increase in the rate of spontaneous transmitter release (miniature end plate potentials frequency). At B, a second perturbation was introduced. The concentration of $[K]_o$ was increased from 2 to 20 mM by iso-osmotic substitution for sodium. This increase in $[K]_o$ is known to produce a depolarisation and an increase in the calcium conductance of nerve membrane¹⁰⁻¹². In normal circumstances, this increase in calcium permeability is expected to cause a calcium influx along the electrochemical gradient for calcium and an increase in transmitter release. At B however, due to the presence of 1 mM EGTA, there is a reversed electrochemical gradient and the increase in calcium permeability presumably causes an efflux of calcium, leading to a decrease in transmitter release. Restoring the inward electrochemical gradient for calcium in C produces a marked increase in m.e.p.p. frequency.

Although extracellular calcium is not necessary to produce hyperosmotic neurosecretion, it has nevertheless an effect on the magnitude of the response. This is illustrated in Fig. 2, where hyperosmotic neurosecretion was induced at A, by adding 50 mM sucrose to standard Ringer solution with 0.4 mM $CaCl_2$. At B the $[Ca]_{out}$

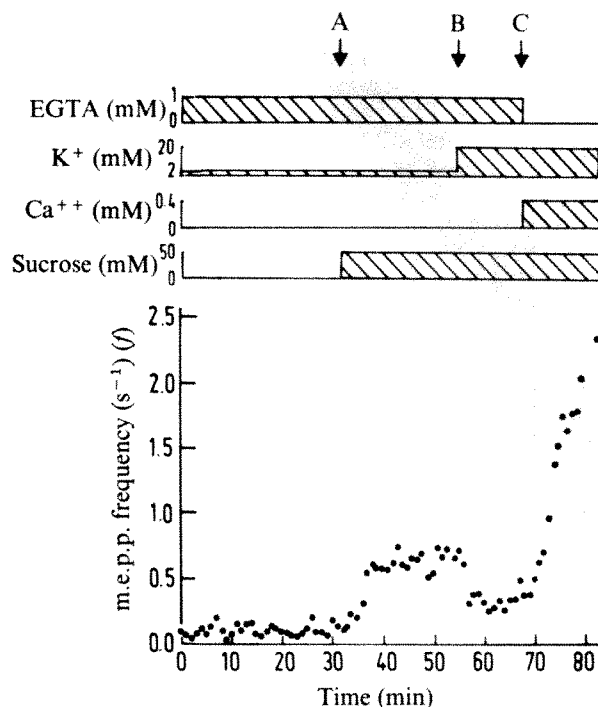


Fig. 1 Hyperosmotic neurosecretion at the frog neuromuscular synapse. A micropipette was placed near the endplate region and the frequency (f) of the spontaneous miniature endplate potentials was monitored at various compositions of the extracellular medium. The control solution contained 116 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1 mM EGTA, with free $[Ca]_o$ of less than 10^{-8} M (with presumably a reversed gradient for calcium ions across the presynaptic membrane). At A the osmolarity of the medium was increased from 225 to 275 mosM, by adding 50 mM sucrose. It is clear that hyperosmotic neurosecretion can be obtained in the virtual absence of calcium ions. At B $[K]_o$ was increased to 20 mM. In contrast to the behaviour in a normal calcium gradient, increase in $[K]_o$ in reversed gradient caused a decrease in f . Restoring an inward calcium gradient in C caused a marked increase in f . Room temperature was approximately 20 °C. The entire experiment was performed at the same fibre, with continuous perfusion of the bathing fluid at an average rate of approximately 2 ml min⁻¹.

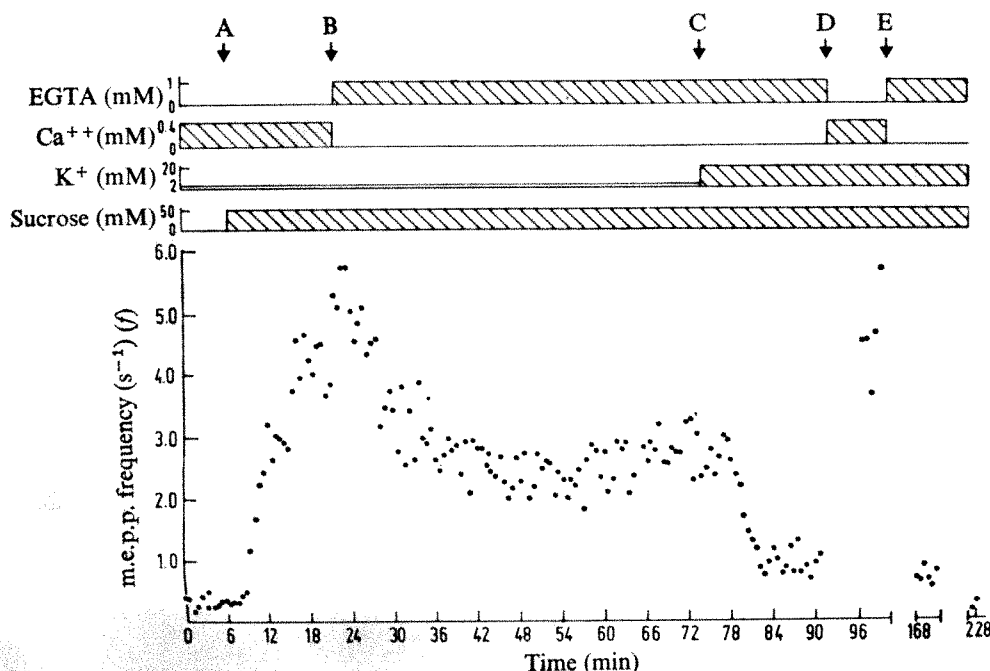


Fig. 2 Factors affecting hyperosmotic neurosecretion at the frog neuromuscular junction. The experiment was started in conditions similar to those in Fig. 1, except that the initial $[Ca]_o$ was 0.4 mM (and no EGTA). Hyperosmotic neurosecretion was produced (at A) by 50 mM sucrose. At B $[Ca]_o$ was reduced to less than 10^{-8} M by adding 1 mM EGTA and omitting $CaCl_2$. Comparison of Fig. 1 and Fig. 2 shows that although $[Ca]_o$ is not necessary for hyperosmotic neurosecretion, it nevertheless influences the magnitude of the response; in the absence of $[Ca]_o$ hyperosmotic neurosecretion is reduced to 40–80%. Addition of $[K]_o$ at C caused again a very substantial decrease in m.e.p.p. frequency. In D and E $CaCl_2$ (0.4 mM) was re-introduced and removed respectively, producing the expected changes in f (see text). Room temperature at 20 °C and continuous perfusion with an average rate of approximately 2 ml min⁻¹.

was reduced to less than 10^{-8} M by omitting calcium and adding 1 mM EGTA; this reduced the m.e.p.p. frequency to approximately 50%. Here again a further decrease was obtained by depolarising the membrane by 20 mM KCl (C).

These experiments suggest that hyperosmotic neurosecretion, similar to other processes which modulate transmitter release^{13,14}, depends on changes in $[Ca]_{in}$. The source for the increase in $[Ca]_{in}$ is not yet clear. One possible origin is the mitochondrion¹⁵, since it has been shown that increase in the osmolarity around liver mitochondria inhibits calcium uptake¹⁶.

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Sodium and calcium action potential in pituitary cells

SEVERAL endocrine cells or their neoplastic derivatives generate action potentials similar to those seen in neurones¹, and in the adrenal chromaffin cell such regenerative potentials depend primarily on a sodium mechanism^{2–4}. Kidokoro has described action potentials in the GH3 rat pituitary cell line which seem to depend on a calcium mechanism⁵. We have re-investigated the action potential in GH3 cells and found that it results from combined Na and Ca mechanisms in physiological conditions. In addition, we have recorded similar electrical excitability from two human pituitary tumours grown *in vitro*.

GH3 cells, provided through the courtesy of Dr Gordon Sato, were dissociated mechanically and grown in 60-mm plastic tissue culture dishes (Falcon) in Dulbecco's MEM with heat-activated serum (5% foetal calf serum (FCS), 10% horse), penicillin (100 U ml⁻¹) and streptomycin (100 µg ml⁻¹). Human pituitary cells were obtained from surgical specimens of an acidophilic adenoma in an acromegalic 51-year-old woman and of a chromophobe adenoma which recurred after surgery and irradiation in a 53-year-old man without endocrine symptomatology. Pieces of tissue were dissociated mechanically or by treatment with 0.027% trypsin for 2.5 h followed by trituration. Dissociated cells were plated in 35-mm Falcon tissue culture dishes (1–6 × 10⁵ cells per dish) which had been coated with gelatine or a

primary culture of cytosine arabinoside-inhibited non-neuronal rat embryo cells. Human pituitary cells were maintained in McCoy's 5A with 20% FCS or MEM with 10% FCS, 200 mg% glucose and antibiotics.

Cells were penetrated with 3.5 M potassium acetate-filled microelectrodes (40–100 MΩ) under guidance of direct vision by phase contrast microscopic control as described previously⁴, except that self-filling microelectrodes were used sometimes. For recording, the cells were bathed in physiological saline consisting of 150 mM Na, 154 mM Cl, 3.5 mM K, 1.8 mM Ca, 1.0 mM Mg and 2.6 mM phosphate (pH 7.3). Occasionally, 2.5 mM Tris buffer was substituted for phosphate buffer.

In most stable and active penetrated GH3 cells, the resting potential reached between –40 and –67 mV, usually after improving from an initially less negative value. The input resistance also increased substantially to values between 40 and 525 MΩ (mean ± s.e. for 13 active cells of 15 with V_m equal to or more negative than –40 mV, 227 ± 38 MΩ). Stimulation of the cells by an intracellular depolarising pulse produced short duration (duration at half height, 2 ms) action potentials (Fig. 1a–c). These occasionally reached the null potential or showed a small overshoot (Fig. 1a). Multiple action potentials could occur in response to a single prolonged stimulus. In cells of 15 with V_m equal to or more negative than injured by impalement, background hyperpolarisation was usually necessary before stimulation could produce an action potential.

Human pituitary cells maintained in culture appeared as small round or spindle shaped cells (Fig. 2a). Cells from both tumours were able to generate action potentials similar to those seen in GH3 (Fig. 2b).

To determine the ionic basis of the electrical activity, cells were first penetrated in control saline, then in

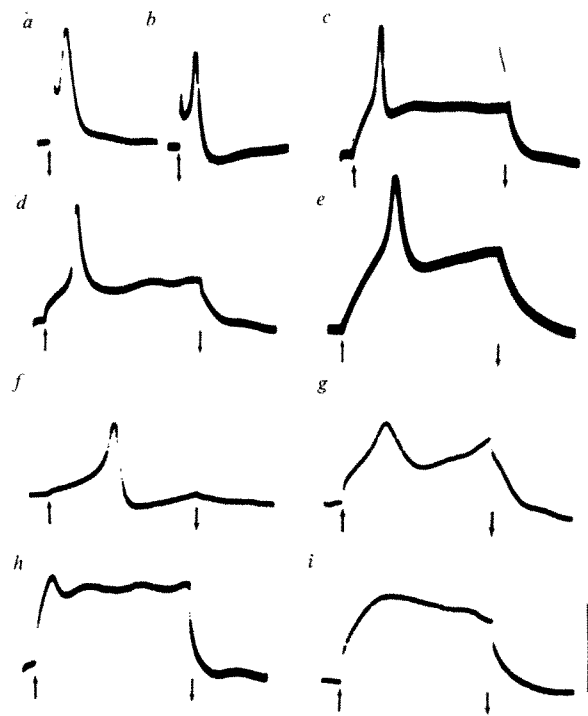


Fig. 1 Action potentials in GH3 cells. a and b, Control medium, 0.5-ms stimulating pulse at double-headed arrows; c, control medium, 50-ms stimulating pulse; d, Ca-free; e, Mn^{2+} ; f, TTX; g, Na-free; h, Ca-free and TTX; i, Mn^{2+} and TTX. Membrane potentials a–i: 52, 56*, 59, 74*, 80*, 46, 54, 80*, 55 mV. (* is V_m with background hyperpolarising current and is only approximate for (d) and (h) due to bridge imbalance.) Stimulus currents c–i: 0.5, 0.4, 1.3, 0.2, 0.6, 0.6 nA. Calibration bars in (i) are 50 mV and 20 ms in all traces.

Table 1 Results of three sets of experiments

a	Control	Ca-free	Ca-free + TTX	Ca-free
No. of active cells/Total no. of cells	14/15	9/9	0/22	10/12
Resting potential (mean $\pm$ s.e.)	22 ± 3 mV	12 ± 1	17 ± 1	16 ± 2
(range)	(11–50)	(10–15)	(11–37)	(10–35)
b	Control	Mn	Mn + TTX	Mn
No. of active cells/Total no. of cells	6/6	7/10	0/16	13/16
Resting potential	32 ± 7	23 ± 3	24 ± 2	25 ± 2
(range)	(16–55)	(12–41)	(12–42)	(16–38)
c	Control	TTX	TTX + Mn	TTX
No. of active cells/Total no. of cells	10/12	8/14	0/12	7/16
Resting potential	22 ± 3	29 ± 4	26 ± 6	25 ± 4
(range)	(10–40)	(10–70)	(10–66)	(12–56)

solutions which either omitted Ca or Na (after four washes), or contained inhibitors of Na or Ca spike mechanisms (tetrodotoxin (TTX) at 10^{-6} g ml $^{-1}$ to inhibit Na spikes, and Mn $^{2+}$ at 5 mM or Co $^{2+}$ at 7.5 mM to inhibit Ca spikes). Chelating agents were not used. Recordings were then made again in control solution. The proportion of cells with maintained resting potentials equal to or more negative than -10 mV which could generate action potentials was determined. The results of three sets of experiments are shown in Table 1, which includes the mean resting potentials ($\pm$ s.e.) and the range of the resting potentials recorded in each condition.

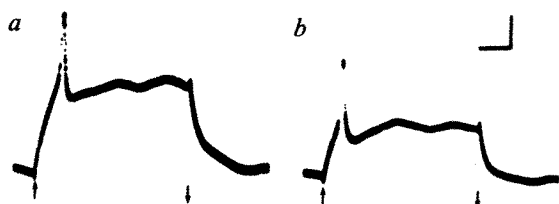


Fig. 2 Human pituitary cells. *a*, Cultured chromophobe adenoma cells seen with phase contrast microscopy after glutaraldehyde fixation. Calibration bar, 50 μ m. *b*, Action potential in control medium from cell from acidophil adenoma. $V_m = 68$ mV, stimulus current = 0.5 nA. *c*, Action potential in Ca-free medium from cell from chromophobe adenoma. $V_m = 80$ mV, stimulus current = 0.7 nA. Calibration = 20 mV and 10 ms.

When Ca was omitted from the medium, action potentials remained frequent and had rates of rise comparable with those in the control (Fig. 1*d*), in spite of lower resting potentials and input resistances. Action potentials were also demonstrated in the presence of 55 mM Mn $^{2+}$ (Fig. 1*e*). To confirm that the spikes recorded in the absence of Ca or in the presence of Mn $^{2+}$ were Na spikes, TTX was added to the Ca-free and Mn $^{2+}$ media. Activity disappeared within minutes (Fig. 1*h*), but could easily be recovered in both cases once the TTX was washed out (Table 1*a* and *b*).

When TTX was added to control saline (1.8 mM Ca), substantial action potentials were still present (Fig. 1*f*). These had lower maximum rates of rise (15 ± 2 V s $^{-1}$ ($n=6$) compared with 58 ± 7 V s $^{-1}$ ($n=13$) in control (active cells with V_m equal to or more negative than -40 mV in both cases)). Rates of rise were measured from photographic records of swept-out action potentials in cells which had been activated maximally by background hyperpolarisation. When Na was replaced in the medium by equiosmolar Tris, action potentials were still found, but with more difficulty (Fig. 1*g*). Spikes were more readily found when Ca was increased to 10 mM. We believe that the difficulty in Na-free conditions was probably due to nonspecific deterioration of the cells. To confirm that the spikes recorded in TTX were Ca spikes, Mn $^{2+}$ was added to the TTX medium. Action potentials promptly disappeared (Table 1*c*), although slight irregularity occasionally remained in the rising phase of the charging curve (Fig. 1*h*).

These experiments demonstrated that the rising phase of the action potential was generated by both a Na and a Ca

mechanism in physiological ionic conditions. The repolarising phase of the action potential could often be seen to undershoot the resting potential (Fig. 1*b*), and reversed at between -50 and -70 mV. If tetraethylammonium chloride (10 mM), an agent known to inhibit the active post-spike K conductance, was added to the medium, the action potential developed a plateau which could last up to 300 ms before repolarisation. It is likely, as in other excitable cells, that the rapid repolarisation of the membrane which terminates the spike is due to an increased K conductance.

The human pituitary tumour studies were limited by the small amounts of tissue in surgical specimens. The cells remained electrically excitable in Ca-free medium (Fig. 2*c*), and in the presence of Co $^{2+}$ (in the one tumour so studied), indicating that a large part of the spike was probably due to Na. The recordings in TTX, however, did not enable us to make a definite statement about the presence or absence of a major Ca component of the spike.

It has been established in various neuronal and endocrine tissues that output of transmitter or hormone is coupled with influx of Ca (ref. 6). In this regard it would seem useful for endocrine cells to utilise Ca-spike mechanisms to enhance excitation-secretion coupling. There is evidence for large Ca-spikes in GH3 cells and pancreatic B cells ^{7,8} but not in adrenal medulla ^{2,3} , pheochromocytoma or bronchial carcinoids ^{1} . In those endocrine cells without large Ca spikes it is likely that Ca entry could occur during depolarisation due to ACh (adrenal medulla) or other stimulatory agents, or during the Na action potential ^{1,9} . The function of Na action potentials in cells with large Ca spikes is less clear. They could help to spread excitation among electrically coupled cells, or they could act in some way as modulators of the Ca mechanism.

The data presented here apply to neoplastic derivatives of anterior pituitary cells. Action potentials have not been reported in normal anterior pituitary cells, but Davis and Hadley ^{10} have recorded extracellular spikes from cells in the pars intermedia of the frog. Preliminary recordings from unidentified rat and gerbil anterior pituitary cells *in vitro* in our laboratory have demonstrated action potentials. The detailed physiology of these responses is yet to be determined.

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Caffeine restores feeding response to 2-deoxy-D-glucose in 6-hydroxydopamine-treated rats

A LARGE portion of the central catecholaminergic nerve terminals of the rat are destroyed by administering 6-hydroxydopamine (6-HDA) via the cerebrospinal fluid¹⁻³. Animals lesioned in this way often appear normal, yet show many subtle behavioural abnormalities⁴. We have been examining one example of this phenomenon, the failure of 6-HDA-lesioned rats to increase food intake when given a systemic injection of 2-deoxy-D-glucose (2-DG) (refs 5, 6). This glucose analogue seems to elicit feeding in intact rats due to its inhibition of glycolysis in cerebral chemoreceptor cells^{7,8}. We have proposed⁹ that lesioned animals do not eat because of an insufficient central catecholaminergic response to the severe decrease in glucose utilisation induced by 2-DG (ref. 10). If so, then pretreatments which serve to augment this neurochemical response might be expected to reinstate behavioural function. Consistent with this hypothesis, very large increases in telencephalic tyrosine hydroxylase activity in 6-HDA-lesioned animals, which occur following chronic insulin treatment, are associated with the restoration of 2-DG-induced feeding¹¹. Many of the physiological effects of catecholamines in the sympathetic nervous system seem to be mediated by an increase in the cyclic AMP concentration of the target cells. Methylxanthenes, such as caffeine and theophylline, inhibit phosphodiesterase, prevent cyclic AMP degradation, and thereby potentiate the catecholamine-stimulated rise in cyclic nucleotide^{12,13}. They also enhance many of the behavioural and physiological effects of catecholamines¹²⁻¹⁵, presumably by the same mechanism. We therefore sought to determine whether the acute administration of those sympathomimetic agents, in intact and 6-HDA-lesioned rats, also would potentiate 2-DG-induced feeding, a behaviour that seems to be mediated, in part, by central catecholaminergic neurons. We report that caffeine restores the 2-DG-induced feeding response.

Male albino Sprague-Dawley rats (Zivic-Miller), weighing 250-300 g at the beginning of each experiment, were housed in individual wire mesh cages and allowed free access to

Table 1 Effect of caffeine and 2-DG on food intake

2-DG dose (mg per kg body weight)	n	Food intake above baseline (g)	
		No caffeine	Caffeine
0	6	-0.9±0.5	0.0±0.4
185	5	+2.2±0.9	+5.1±0.5
375	5	+3.2±0.8	+5.2±0.8
750	5	+3.6±0.5	+7.2±1.4

Food intake was measured every hour for 5 h (9.30 a.m. until 2.30 p.m.) on each of two successive days. On day 3, after the first hour of testing, each rat was injected i.p. with isotonic saline or 2-DG. Animals given 2-DG consumed significantly more food (mean±s.e.) during the following 4 h than they had on previous days ($P < 0.01$ in each case). One week later, the rats were tested as before, except that on day 3 they were given caffeine (50 mg per kg, i.p.) along with saline or 2-DG. Rats given 2-DG consumed even more food than they had when 2-DG alone was given ($P < 0.05$ in each case).

Table 2 Effect of xanthenes on 2-DG-induced feeding

Xanthene dose (mg per kg body weight)	n	Food intake (g)	
		Baseline	2-DG + xanthene
None	5	1.3±0.5	3.8±0.7
Caffeine			
12.5	5	0.8±0.3	5.9±0.7
25.0	5	0.9±0.5	6.9±0.5
50.0	5	1.0±0.5	6.3±1.1
100.0	3	0.9±0.5	1.5±0.4
Theophylline			
12.5	5	1.3±0.5	6.9±0.4

Food intake (mean±s.e.) was determined according to the protocol described in Table 1, except that the dose of 2-DG was held constant (375 mg per kg, i.p.) and either the dose of caffeine was varied or theophylline was given instead. All but the highest dose of caffeine increased food intake significantly more than when 2-DG alone was given ($P < 0.01$ in each case).

Purina Lab Chow pellets and tap water. In the first series of experiments animals were given 2-DG, either caffeine or theophylline, or a combination of 2-DG and a xanthene. 2-DG added alone produced a dose-dependent increase in food intake (Table 1). Neither caffeine nor theophylline affected food intake when given alone, but both significantly enhanced the feeding response to 2-DG (Tables 1 and 2).

In the second series of experiments, we administered 6-HDA (200 µg in 20 µl) or its vehicle (0.9% NaCl, 0.1% ascorbic acid) by way of the lateral ventricle 30 min after an intraperitoneal (i.p.) injection of desmethylinipramine (25 mg per kg body weight). We have previously found this treatment to produce a permanent 90-95% depletion of telencephalic dopamine, without altering brain noradrenaline content⁶. Two to three weeks after the lesion was produced, the effects on food intake of optimal doses of 2-DG (375 mg per kg, i.p.) and caffeine (25 mg per kg, i.p.) were determined.

Table 3 Effect of caffeine and 2-DG on food intake in intact and 6-HDA-lesioned rats

	Food intake (g)	
	Intact	6-HDA
Baseline	1.1±0.3	1.1±0.4
2-DG	4.4±1.3	0.7±0.4
2-DG + caffeine	7.7±0.6	7.2±1.0

Food intake (mean±s.e.) was determined according to the protocol described in Table 1, except that a single dose of 2-DG (375 mg per kg, i.p.) was given with caffeine (25 mg per kg, i.p.). Intact rats ($n = 7$) ate more when 2-DG was given with or without caffeine ($P < 0.05$ and < 0.001 , respectively), whereas 6-HDA-lesioned rats ($n = 7$) only ate more when 2-DG was given with caffeine ($P < 0.001$).

Vehicle-treated rats increased food intake in response to 2-DG, and ate even more when caffeine was also administered (Table 3). As expected, 6-HDA-lesioned rats did not increase their food intake after 2-DG treatment alone. When 2-DG was administered together with caffeine, however, the lesioned rats ate significantly more food than before, almost as much as controls did in response to the same treatment (Table 3).

There are several possible explanations for the effects of caffeine on 2-DG-induced feeding in control and lesioned animals. First, caffeine may have altered the effectiveness of 2-DG as a competitive inhibitor by somehow reducing the concentration of circulating glucose. This is apparently not the case, however, since caffeine, if anything, increased the normal hyperglycaemic response to 2-DG (Table 4). Second, because of the ubiquitous involvement of cyclic AMP as a 'second messenger', caffeine may be acting by potentiating the effects of some hormone or transmitter other than brain catecholamines. While the present data do not rule out this possibility, there is a good reason to

believe that 2-DG-induced feeding is mediated in part by the release of central amines since it can be blocked both by the selective destruction of catecholaminergic terminals^{5,6,16} and by AMT (ref. 16), an inhibitor of catecholamine biosynthesis. Third, and most likely, caffeine may produce a net increase in the activation of target cells by catecholamines. This may occur either by potentiating the effects of catecholamines on cyclic AMP levels or by increasing catecholamine release, another known property of methylxanthenes¹⁷. Experiments to further elucidate the mechanisms of this phenomenon are in progress.

Table 4 Effect of caffeine and 2-DG on blood glucose concentration

2-DG dose (mg per kg body weight)	Blood glucose concentration (mg per 100 ml)	
	No caffeine	Caffeine
0	114 ± 5	124 ± 7
185	153 ± 15	201 ± 7
375	274 ± 16	288 ± 14

Blood glucose concentration (mean ± s.e.) in rats after 0 or 25 mg per kg caffeine was given i.p. along with isotonic saline or 2-DG. Food was withheld for 1 h, after which rats were killed by decapitation. From blood samples obtained when rats were killed, glucose was determined colorimetrically following its reaction with glucose oxidase (Worthington) ($n = 4$ or 5 in each group). Statistically significant effects of caffeine were observed only when 185 mg per kg 2-DG had been administered ($P < 0.05$).

Our data are consistent with the hypothesis that 6-HDA-lesioned rats fail to respond behaviourally to 2-DG because of an inadequate activation of central catecholamine receptors. Previously, we also have found that amphetamine will stimulate feeding in anorexic 6-HDA-treated rats⁴, and comparable results have been obtained by others^{18,19}. It is of interest that caffeine and amphetamine both have been used in the treatment of such seemingly diverse syndromes as narcolepsy²⁰ and hyperkinesis^{21,22}. The ability of the sympathomimetic drugs to restore function following damage to central catecholaminergic systems suggests that similar damage may be present in these clinical disorders^{23,24}.

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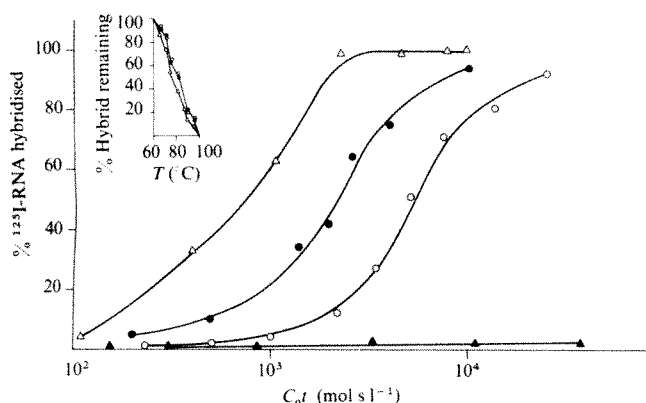
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Nucleic acid sequences of primate type C viruses in normal and neoplastic human tissues

RECENT reports on the isolation of type C viruses from human cells have generated considerable controversy because of the close relationship of the viruses to the type C viruses of subhuman primates^{1–5}. These findings have been interpreted as evidence for contamination despite the repeated isolation of some type C viruses from separate clinical specimens or from separate frozen primary cell stocks of the same donor^{6–9}. Our laboratory has described a type C virus which is released from the HEL-12 strain of normal human embryonic lung fibroblasts⁵. The HEL-12 virus is related immunologically to simian sarcoma (woolly monkey fibrosarcoma) virus (SiSV) and the endogenous type C viruses of baboons and domestic cats^{9–11}. To demonstrate that the HEL-12 virus is not an adventitious contaminant, fresh human tissues were examined for antigenic cross-reactivity with HEL-12 virus antigens. Some patients with myelogenous leukemia contain circulating immunoglobulins which specifically inhibit the reverse transcriptases of HEL-12 virus and primate type C viruses¹². In addition, the glomerular immune complexes in patients with systemic lupus erythematosus contain antigens related to HEL-12 virus proteins¹³. Using the techniques of molecular hybridisation, we demonstrate here that HEL-12 cells contain proviral DNA sequences before antigen expression or spontaneous virus release can be detected. We also present evidence for the existence of nucleic acid sequences homologous to HEL-12 viral RNA in the DNAs from certain cancer patients.

HEL-12 cultures were grown from frozen primary cell stocks⁵. Type C viral antigens or extracellular virus were not detected during the first 20–40 d, but the cultures gradually expressed cytoplasmic viral antigens and released type C particles between 80 and 120 d. DNA was extracted from early-passage (36 d of culture) HEL-12 cells in which type C viral antigens were not detected by indirect immunofluorescence or from late-passage (115 d of culture) HEL-12 cells which spontaneously released type C virus^{9,14}. The DNA was sheared, denatured and reannealed in the presence of trace amounts ¹²⁵I-HEL-12 viral RNA. Aliquots were evaluated for duplex formation at various times thereafter by observing resistance to pancreatic RNase digestion in conditions of high ionic strength. Both early-passage and late-passage HEL-12 cells contained DNA sequences homologous to HEL-12 viral RNA (Fig. 1). The relative displacement of the curves along the abscissa suggested that early-passage cells contained fewer copies of the viral genome than late-passage cells. The ¹²⁵I-HEL-12 RNA also hybridised to DNA from a marmoset fibroblast line infected with SiSV (71AP1/SiSV), thereby confirming the relatedness of HEL-12 virus to SiSV⁵. The ¹²⁵I-HEL-12 viral RNA probe eventually hybridised to a similar extent with DNA from early-passage and late-passage HEL-12 cells and 71AP1/SiSV cells. Some differences were noted between the t_m values of the hybrids formed with HEL-12 viral DNAs and 71AP1/SiSV-DNA, suggesting that the proviral sequences being detected were not identical in all cases (Fig. 1, Table 1). Since HEL-12 cell cultures express antigens related to SiSV and baboon endogenous virus (BaEV)^{9,14}, HEL-12 viral RNA was hybridised to DNA extracted from a bat lung cell line productively infected with BaEV. The stock of HEL-12 viral RNA used for these experiments lacked detectable BaEV sequences (Table 1), possibly because the HEL-12 cell cultures released virions with SiSV in great excess. Moreover, we have observed intervals in the lifespan of HEL-12 cells during



(Grade T-2, Collaborative Research) was added. The mixture was stirred at room temperature for 16 h. The oligo(dT)-cellulose was removed and packed into a Pasteur pipette which was washed exhaustively with $2 \times \text{SSC}$. The poly(A)-containing HEL-12 viral RNA was eluted by successive washes of the column with 10 mM Tris-HCl, pH 7.2. The eluate was adjusted to a final NaCl concentration of 0.25 M in order to stabilise the RNA. Iodination was performed as originally described by Commerford¹⁸ and modified by Prenskey *et al.*¹⁰. The specific activity of the ^{125}I -RNA was approximately $5\text{--}10 \times 10^7$ c.p.m. per μg . Hybridisations were carried out in sealed glass capillary tubes with approximately 1,000 c.p.m. of ^{125}I -viral RNA in 0.4 Na-phosphate, pH 6.8. The DNA-RNA ratio in all cases was at least 5×10^6 . Each reaction mixture was placed in boiling water for 10 min and subsequently incubated at 60°C . Hybrids were evaluated at progressively greater C_0t values²⁰ by determining resistance to $20 \mu\text{g ml}^{-1}$ pancreatic RNase (Worthington) at high ionic strength ($2 \times \text{SSC}$) for 2 h at 37°C . Less than 3% of the ^{125}I -viral RNA was resistant to RNase when incubated in the absence of DNA or when incubated to $C_0t = 4 \times 10^4$ with calf thymus DNA. This resistant fraction was subtracted from all values. The C_0t values shown are uncorrected for $[\text{Na}^+]$ ion concentration. The final extent of hybridisation is plotted relative to an absolute value of 43% obtained using 71API/SiSV DNA. Inset, thermal stabilities of ^{125}I -RNA:DNA hybrids. ^{125}I -HEL-12 viral RNA and DNAs from various sources were annealed to a C_0t of $10^4\text{--}2 \times 10^4$ and diluted into 50–100 volumes of $1 \times \text{SSC}$ buffer. Aliquots were held at the indicated temperatures for 10 min, adjusted to a final concentration of $2 \times \text{SSC}$ buffer and digested with $20 \mu\text{g ml}^{-1}$ pancreatic RNase for 2 h at 37°C . Sources of DNAs: Δ , marmoset fibroblast cells releasing SiSV (71API/SiSV); $\circ$, HEL-12 cells passaged for 36 d; $\bullet$, HEL-12 cells passaged for 115 d; $\blacktriangle$, calf thymus DNA.

which only SiSV-related antigens are expressed¹⁴. The absence of BaEV-RNA in this stock of HEL-12 virus has been confirmed by R. G. Smith and R. C. Gallo (personal communication).

Two of 14 DNA samples from nine cancer patients likewise contained DNA sequences hybridisable to the RNA of HEL-12 virus (Fig. 2). In one case, proviral sequences were detected in the spleen of an 11-yr old boy who died from an osteogenic sarcoma. This tumour uncommonly metastasises to the spleen²¹, and no evidence for splenic metastases was obtained by histological post-mortem examination. The results with DNAs from other tissues of this patient were interesting because HEL-12 proviral sequences were not detected in the primary tumour from the femur

or in the patient's liver (Fig. 2). A second case involved the spleen of an adult with acute myelogenous leukaemia. Proviral DNA sequences were found in the spleen although to a lesser extent than in early-passage or late-passage HEL-12 cells or in the spleen of the boy with osteogenic sarcoma (Fig. 2). The thermal stabilities of these hybrids were not appreciably different from those of the ^{125}I -RNA:HEL-12 DNA hybrids (Fig. 2, Table 1).

The results (Table 1) can be summarised as follows: (1) DNA extracted from early-passage HEL-12 cells contain proviral sequences. (2) With continued *in vitro* propagation, HEL-12 cells acquire an increased complement of proviral sequences, possibly as the result of a selective proliferation of a cell fraction containing the HEL-12 viral genome or

Table 1 HEL-12 proviral sequences in various DNAs

Diagnosis	Source of DNA	Final relative % hybridisation of ^{125}I -HEL-12 viral RNA	Hybrid t_m ($^\circ\text{C}$)	Δt_m ($^\circ\text{C}$)
—	71API/SiSV	100	77	8.0
—	HEL-12 cells, early-passage	95	82.5	2.5
—	HEL-12 cells, late-passage	93	82.0	3.0
Osteogenic Sarcoma	Spleen	90	84.5	0.5
	Liver	1	—	—
	Primary tumour	1	—	—
AML	Spleen	61	85.0	0
	Liver	2	—	—
AML	Spleen	4	—	—
AML	Liver	3	—	—
AML	Kidney	2	—	—
	Liver	2	—	—
AML	Peripheral leukocytes	1	—	—
AML	Peripheral leukocytes	3	—	—
ALL	Kidney	2	—	—
Nasopharyngeal Carcinoma	Liver	4	—	—
	Spleen	0	—	—
—	BL88/BaEV	6	—	—

The actual % hybridisation of 71API/SiSV DNA was 43. The Δt_m ($^\circ\text{C}$) is expressed relative to a t_m of 85.0°C for ^{125}I -HEL-12 viral RNA: AML spleen DNA hybrid. AML, acute myelogenous leukaemia; ALL, acute lymphatic leukaemia; BL88/BaEV, bat lung cells infected with BaEV from *Papio anubis*. All DNAs were annealed with ^{125}I -HEL-12 viral RNA to a $C_0t \geq 10^4$.

from a low level of virus spread from cell to cell in early-passage cultures. Embryonic tissues were not available to demonstrate proviral sequences in the original lung tissue from which the HEL-12 cell strain was established in 1974. Even if available, proviral sequences might not be found if present in a small number of cells of such heterogeneous tissues. (3) DNA sequences homologous to HEL-12 viral RNA have also been detected in the spleen DNA of a boy with osteogenic sarcoma, and to a lesser extent, in the spleen of an adult with acute myelogenous leukaemia. The proviral sequences in the DNAs of HEL-12 cells and fresh human tissues were related to SiSV as evidenced by the ability of the ^{125}I -HEL-12 viral RNA to hybridise to 71AP1/SiSV DNA. Thermal denaturation profiles of these hybrids, however, reveal some differences between the hybrids formed between HEL-12 viral RNA and DNA from cells producing HEL-12 virus or SiSV. This suggests

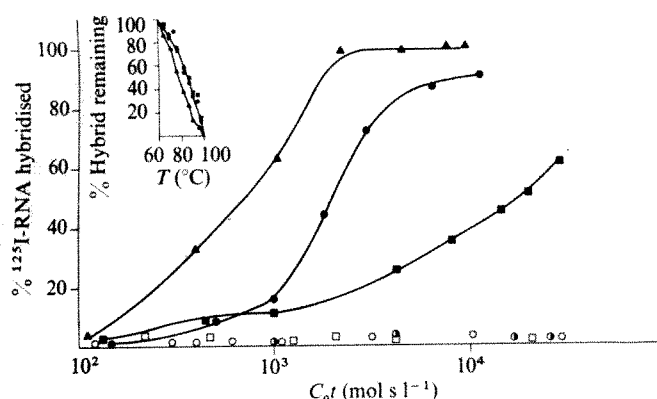


Fig. 2 Hybridisation of ^{125}I -HEL-12 viral RNA to DNAs from organs of cancer patients. The conditions for purification and shearing of DNAs were as described in Fig. 1. The final extent of hybridisation is plotted relative to that obtained with 71AP1/SiSV DNA. Source of DNAs: Δ , 71AP1/SiSV (from Fig. 1); $\bullet$, spleen, and $\square$, liver from patient with osteogenic sarcoma; $\circ$, primary osteogenic sarcoma; $\blacksquare$, spleen and $\square$, liver from patient with acute myelogenous leukaemia. Inset, thermal stabilities of hybrids in $1\times\text{SSC}$ buffer.

that the proviral sequences in HEL-12 cells and fresh human tissues may not be identical to those detected in 71AP1/SiSV cells. The presence of proviral sequences in early-passage HEL-12 cells argues against the notion that the HEL-12 virus is a laboratory contaminant, especially since primate type C viruses have not been propagated by us³. But, by far the strongest argument against adventitious contamination seems to be the presence of SiSV-related sequences in fresh autopsy tissues of patients with malignancies.

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Chaos in an enzyme reaction

DYNAMIC systems are usually thought to have either monotonic or periodic behaviour. Although the possibility of other types of behaviour has been recognised for many years, the existence of non-monotonic, non-periodic behaviour in dynamic systems has been firmly established only recently. It is termed chaotic behaviour. A review on the rapidly expanding literature on chaos in discrete model systems described by difference equations has been published by May¹. Rössler², on the other hand, has discussed a few published works on systems of differential equations with chaotic solutions, and he has proposed a three-component chemical model system which he argues has chaotic

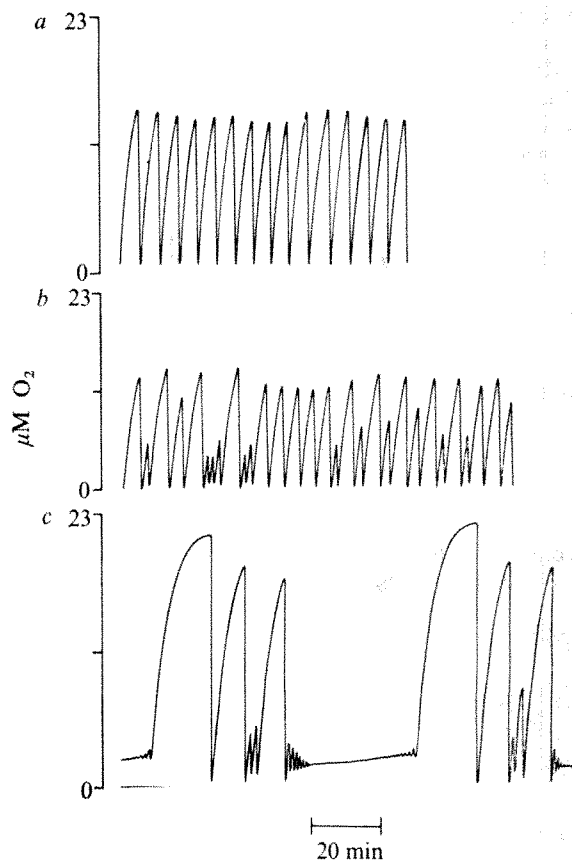


Fig. 1 Oscillations in oxygen concentration in peroxidase catalysed oxidation of NADH in system open to O_2 . A stirred 5 ml sample containing horseradish peroxidase, 10 μM 2,4-dichlorophenol and 0.2 μM methylene blue in a 0.1 M Na-acetate buffer pH 5.1 was in contact with an O_2/N_2 gas mixture at atmospheric pressure containing 1.9 volume % O_2 . A solution of 0.2 M NADH was pumped into the sample at 11 μl per h. The oxygen concentration in the liquid was measured with a Clark electrode. The temperature was 28 $^\circ\text{C}$. The concentration of peroxidase was a, 0.90 μM ; b, 0.55 μM and c, 0.45 μM .

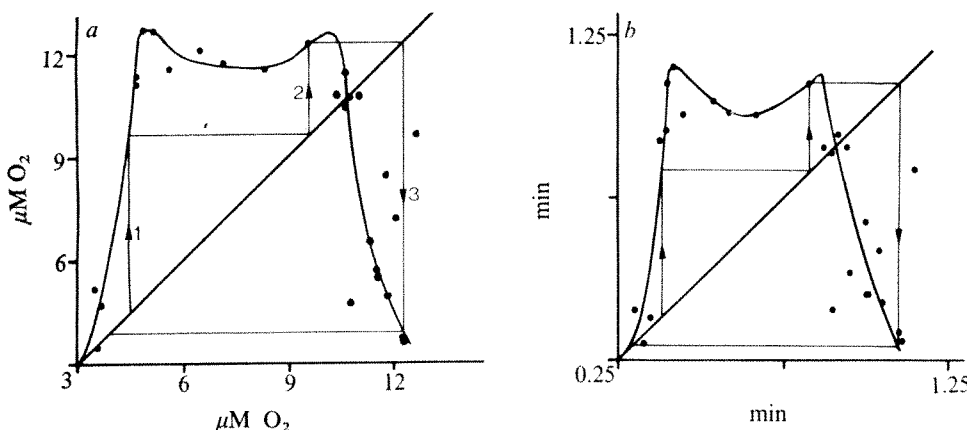


Fig. 2 a, Amplitudes ($A(n+1)$) plotted against preceding amplitudes ($A(n)$) from Fig. 1b. b, Periods ($P(n+1)$) plotted against preceding periods ($P(n)$) from Fig. 1b. Trajectories with arrows were drawn to show that the transition functions allow the period 3.

solutions. The argument is based on a theorem by Li and Yorke³. Here we report the finding of chaotic behaviour as an experimental result in an enzyme system (peroxidase). Like Rössler² we base our identification of chaos on the theorem by Li and Yorke³.

When the oxidation of NADH by O_2 , catalysed by horse-radish peroxidase, takes place in a stirred solution where O_2 can enter by diffusion from the gas phase, oscillations^{4,5} and bistability⁶ may be observed in the reaction system. Sustained oscillations may be achieved by continuously supplying NADH to the reaction mixture. In the present experiments this was done by pumping in an NADH solution at a slow constant rate. The wave form of the oscillation depended on the enzyme concentration as shown in Fig. 1. In Fig. 1b there is no apparent periodicity. This irregular oscillation was analysed by plotting each amplitude against the preceding amplitude as described by Lorenz⁷. This plot (Fig. 2a) reveals that the amplitudes found in our experiment satisfy a well defined cap-shaped transition function. When the durations of the periods are plotted in the same way a similar transition function is found (Fig. 2b). A random distribution of points in the plane would have been expected if the irregularities in the oscillation were caused by random perturbations.

The existence of the cap-shaped transition function indicates that the system has either a periodic behaviour with a complicated period or it has chaotic behaviour. The two possibilities can be distinguished using the theorem of Li and Yorke³ which states that if the transition function allows the period three it allows any period and chaos exists. In Fig. 2a and b it is seen that it is possible to design trajectories of three transitions which will bring the system back beyond the origin. Thus our system exhibits chaotic behaviour in the Li and Yorke sense. We are not aware of any other examples of chaos in chemical reaction systems.

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Specific inhibition of chromatin-associated poly(A) synthesis *in vitro* by cordycepin 5'-triphosphate

It has been established that many eukaryotic mRNAs contain poly(adenylic acid) tracts at their 3'-termini. The polyadenylation of mRNA occurs post-transcriptionally in the nucleus as a rapid, initial addition of 100–200 adenylate residues to the pre-mRNA (ref. 1). Subsequently, a slower chain extension (6–8 bases) of the poly(A) tail seems to occur both in the nucleus and in the cytoplasm². The initial polyadenylation reaction can be specifically inhibited by the drug cordycepin (3'-deoxyadenosine) in cell culture, presumably by its conversion to the triphosphate analogue which acts as a competitive inhibitor of poly(A) polymerase. Cordycepin, however, has little effect on the slower poly(A) extension reaction³ or on the formation of mRNA precursor molecules^{4–6}; but it can inhibit rRNA synthesis⁷. Contrary to the *in vitro* observations, cordycepin 5'-triphosphate (3'dATP) is not a specific inhibitor of poly(A) synthesis *in vivo*, relative to RNA synthesis^{8,9}, and RNA polymerase I (which synthesises rRNA) is actually less sensitive to inhibition by 3'dATP than RNA polymerase II (ref. 10) (which is presumed to be involved in the synthesis of mRNA). Since nuclear poly(A) polymerase occurs in two functional states as 'free' and 'chromatin-bound' forms¹¹, we reasoned that if the chromatin-associated poly(A) polymerase were involved in the initial polyadenylation of mRNA, it might be selectively inhibited by 3'dATP. The present studies, designed to test such an idea, demonstrate that, as *in vivo*, the initial polyadenylation reaction can be selectively inhibited *in vitro* by low levels of 3'dATP. These data also show that higher levels of 3'dATP can inhibit RNA synthesis, 'chromatin-bound' RNA polymerase I activity being significantly more sensitive than the 'bound' RNA polymerase II activity.

Initial studies measuring the effect of 3'dATP on poly(A) synthesis catalysed by 'bound' and 'free' poly(A) polymerases in whole nuclei, indicated that the 'bound' enzyme was 100–200 times more sensitive than the 'free' poly(A) polymerase. To study the inhibition by 3'dATP in more detail, we separated the poly(A)-synthesising activities of isolated nuclei by preparing a nuclear sap fraction and chromatin from the nuclear residue to yield the 'free' and 'bound' enzymes, respectively. The effect of increasing concentrations of 3'dATP on poly(A) synthesis catalysed by the 'free' and by the 'bound' enzyme is shown in Fig. 1a. Approximately 150 $\mu\text{g ml}^{-1}$ of drug were needed to achieve 50% inhibition of the 'free' enzyme, whereas the same inhibition was attained with less than 1 $\mu\text{g ml}^{-1}$ of 3'dATP

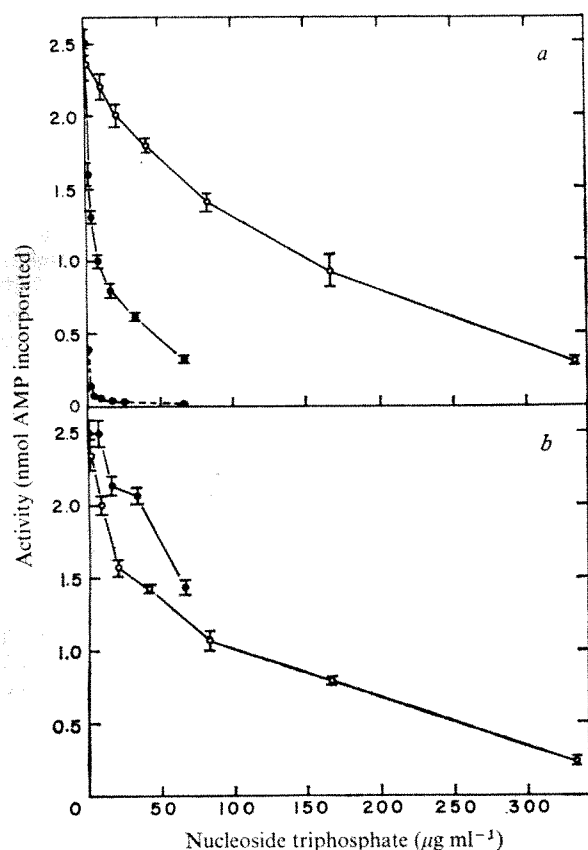


Fig. 1 Effect of 3'dATP (a) and 2'dATP (b) on 'free' and 'bound' poly(A) polymerases. Rat liver nuclei were prepared by homogenisation of the tissue in hypertonic sucrose (2.3 M) (ref. 15) and the isolated nuclei were gently resuspended in hypotonic buffer essentially as described previously¹¹. After centrifugation (40,000g; 30 min) to remove the nuclei, glycerol was added to the supernatant fraction so that the final concentration was 20% (v/v). This nuclear supernatant fraction was the source of the 'free' poly(A) polymerase. Chromatin was prepared from the nuclear pellet after washing with the same hypotonic buffer. Isolation of chromatin was as described previously¹⁶ except that the Triton X-100 and NaCl washes were omitted. The final chromatin pellet was resuspended in 0.4 mM Tris-HCl (pH 8) (0.5 ml per g liver) and used within 36 h of preparation. The DNA content of nuclei and chromatin was estimated as described by Burton¹⁷. Poly(A) polymerase activity was assayed in a reaction mixture containing 38 mM Tris-HCl (pH 8), 38 mM KCl, 0.7 mM MnCl₂, 1.0 mg per ml phosphoenolpyruvate, 2 μg per ml pyruvate kinase, 0.37 mM ³H-ATP (2,000 c.p.m. nmol⁻¹), addition of 3'dATP or 2'dATP as indicated and appropriate amounts of poly(A) polymerase in a total volume of 120 μl. For analysis of 'free' poly(A) polymerase, poly(A) was included at a concentration of 375 μg ml⁻¹. After 1 h at 37 °C, the reaction was terminated by addition of excess ATP (10 μl of ATP, 10 mg ml⁻¹). Aliquots of 110 μl were spotted on to DE81 filters and processed as described previously¹⁵. All assays were performed in quadruplicate; zero time backgrounds were less than 150 c.p.m. ○, 'Free' poly(A) polymerase obtained from nuclei representing 45 μg DNA; ●, chromatin representing 50 μg (---) or 160 μg (—) DNA. Horizontal bars indicate s.e.m.

when 'bound' poly(A) polymerase was derived from an equivalent amount of tissue. Since the activity catalysed by the chromatin-associated enzyme represents only a small percentage of the activity of the 'free' poly(A) polymerase, we also measured the effect of 3'dATP when the levels of chromatin and nuclear supernatant (sap) fractions were adjusted to give the same activity. When the activities were so adjusted, the 'bound' enzyme was still 75 times more sensitive than the 'free' enzyme.

In order to further substantiate the specific inhibitory effect of 3'dATP on chromatin-associated poly(A) polymerase relative to 'free' enzyme, the effect of 2'dATP, the naturally occurring deoxynucleotide, was measured. 2'dATP

did not specifically inhibit chromatin-bound poly(A) synthesis (Fig. 1b); in fact, 'free' poly(A) polymerase was slightly more sensitive to inhibition by 2'dATP than the 'bound' enzyme. Interestingly, inhibition of 'free' poly(A) polymerase by 2'dATP was greater than that obtained with 3'dATP, 50% inhibition occurring at 70 and 120 μg ml⁻¹, respectively.

Since 3'dATP is an ATP analogue, the relationship of the inhibitor to substrate levels was investigated. Figure 2 shows the double reciprocal plots of velocity against ATP concentration in the presence and absence of 3'dATP. The inhibition of the 'free' poly(A) polymerase by 3'dATP was competitive with substrate (b). The K_m and K_i calculated from Fig. 2b were 62 μM and 40 μM, respectively. When the relationship of 3'dATP to ATP levels was investigated using the chromatin fraction, a non-competitive relationship was observed (Fig. 2a). The calculated K_m and K_i were 100 μM and 1.3 μM, respectively. Although the K_m values of the two enzyme activities were similar, the K_i for chromatin-associated poly(A) synthesis was much lower, indicating that this reaction was at least 30 times more sensitive to inhibition by 3'dATP than poly(A) synthesis by the 'free' enzyme. Inhibition of poly(A) polymerase for both the 'free'

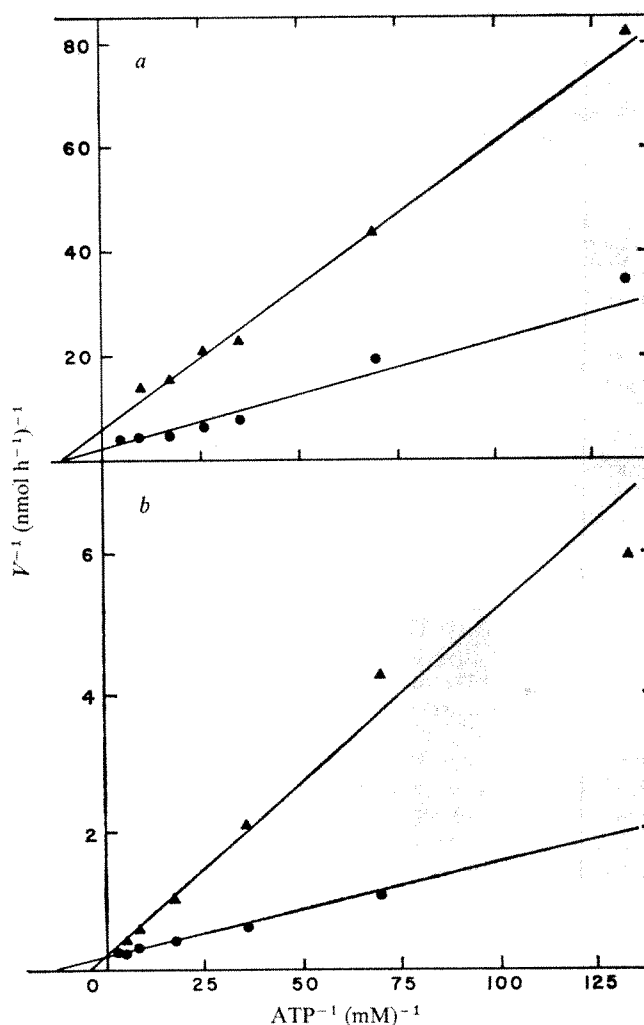


Fig. 2 Effect of 3'dATP on the double reciprocal plot of velocity against substrate concentration for 'bound' and 'free' poly(A) polymerases. Chromatin and nuclear supernatant fractions were prepared and assayed as described in Fig. 1 in the presence (▲) and absence (●) of 3'dATP at varying ³H-ATP⁻¹ levels. a, Chromatin equivalent to 60 μg DNA and 3'dATP at a concentration of 4 μM were used; b, 'free' poly(A) polymerase was derived from 90 μg nuclear DNA and 3'dATP was at a concentration of 118 μM. The ATP concentration in the assay ranged from 7–235 μM with specific activities ranging from 2 × 10⁵ to 7 × 10⁵ c.p.m. nmol⁻¹. Lines were fitted by least squares analysis.

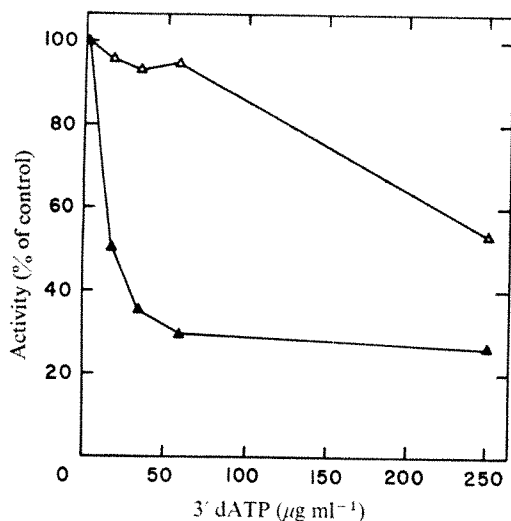


Fig. 3 Effect of 3'dATP on chromatin-bound RNA polymerases. RNA synthesis was measured in an assay similar to that described in Fig. 1 except that CTP, GTP and ^3H -UTP (30 c.p.m. pmol $^{-1}$) were included at a concentration of 50 μM . Chromatin equivalent to 60 μg DNA was used. Reactions were incubated for 5 min at 30 °C, after which excess UTP was added and filters processed as described previously¹⁵. Reactions were performed in quadruplicate; zero times backgrounds were less than 100 c.p.m. Analyses were performed in the presence and absence of α -amanitin. The decrease in activity when 1 $\mu\text{g ml}^{-1}$ α -amanitin was included represented RNA polymerase II. Activity in the presence of α -amanitin represented primarily RNA polymerase I since high levels of toxin (150 $\mu\text{g ml}^{-1}$) produced only 10–15% decrease in activity (indicating little RNA polymerase III contamination). Δ , RNA polymerase II, 100% activity corresponded to 22.8 ± 1.8 pmol UMP incorporated in 5 min; $\blacktriangle$, RNA polymerase I, 100% activity corresponded to 13.2 ± 0.8 pmol UMP incorporated in 5 min.

and 'bound' forms by 2'dATP was competitive with ATP levels, so it seems that not only is 3'dATP a specific inhibitor of chromatin-associated poly(A) synthesis, but that its mode of action differs from inhibition of the 'free' form of enzyme.

We then measured the effect of 3'dATP on RNA polymerase activity associated with the chromatin fraction, maintaining the assay conditions as close as possible to those for poly(A) synthesis to avoid unnecessary artefacts. Figure 3 shows the inhibition of chromatin-associated RNA polymerases I and II by 3'dATP. It can be seen that polymerase I was more sensitive to the inhibitor than polymerase II. At 60 $\mu\text{g ml}^{-1}$ 3'dATP, which inhibited polymerase I activity by 70%, polymerase II activity was inhibited only by 5%. Although the 'bound' polymerase I activity was reduced by 3'dATP, this inhibition was far less than observed for 'bound' poly(A) polymerase activity, where 50% inhibition could be achieved at concentrations of less than 1 $\mu\text{g ml}^{-1}$ using a similar amount of chromatin. RNA polymerase activity can also be detected in the nuclear supernatant fraction¹². As previously reported for partially purified enzymes^{8,10}, 3'dATP had a similar effect on 'free' RNA polymerases present in the nuclear supernatant fraction, with a dose-response curve similar to that for chromatin-bound RNA polymerase I (data not shown).

The marked sensitivity of chromatin-associated poly(A) polymerase, the lack of inhibition of RNA polymerase II in the same subnuclear fraction and the intermediate sensitivity of chromatin-bound RNA polymerase I by 3'dATP are results similar to those obtained *in vivo* by cordycepin. The lack of differential inhibition of 'free' poly(A) polymerase relative to 'free' RNA polymerases by 3'dATP supports the observations made using partially purified enzymes. Thus, although purified enzymes are invaluable in determining reaction characteristics, data obtained from such systems

should be interpreted with caution. In this case, inhibition of partially purified enzymes by 3'dATP, probably reflects the effects of the drug on extrachromosomal events. The selectivity of inhibition of chromatin-associated poly(A) synthesis by 3'dATP is further evident when a comparison is made with the effect of the compound on polyadenylation of endogenous RNA in isolated mitochondria, where chromatin factors are not present. Inhibition of the mitochondrial polyadenylation reaction occurs only at relatively high concentrations of 3'dATP (ref. 13) (50% inhibition occurring at 300 μM , a value even greater than the K_m). The lack of inhibition of mitochondrial polyadenylation at this concentration of 3'dATP agrees well with the relative insensitivity of mitochondrial polyadenylation to cordycepin *in vivo*¹⁸.

The remarkable sensitivity of chromatin-associated poly(A) polymerase to cordycepin triphosphate, the close association of this activity with DNA and the similar inhibition responses of the initial polyadenylation reaction *in vivo* and 'bound' enzyme activity *in vitro*, all tend to indicate that the latter activity indeed represents the initial polyadenylation reaction. The 'free' poly(A) polymerase activity, by virtue of its localisation in the nuclear supernatant and cytosol fractions¹⁴, coupled with its relative insensitivity to low levels of 3'dATP, should represent the enzyme activity responsible for the additional polyadenylation reaction. These studies have thus provided a simple system to differentiate between initial polyadenylation and subsequent chain extension reactions *in vitro*.

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matters arising

The Late Weichselian geomagnetic event

THOMPSON and Berglund¹ describe palaeomagnetic results from Björkeröds Mosse which, they claim, invalidate all previous evidence for a Late Weichselian geomagnetic event. In this note I discuss these results and answer their criticisms in regard to evidence which I have presented².

Thompson and Berglund stress the difficulty of interpreting palaeomagnetic records from sedimentologically complex cores and yet the 'carefully selected site' at Björkeröds Mosse contains eight sedimentary units from three climatic zones. The high noise content of the magnetisation, evident in Fig. 1 of ref. 1, thus supports their contention that variable sediments are poor recorders of the ambient field and this is confirmed by the absence of any secular variation periodicity comparable with cycles in archaeomagnetic data³. Their scattered directions have probably arisen from the spasmodic growth of post-depositional magnetisation, while the general decrease in inclination with depth through layers 8 to 2 is consistent with increasing compaction in these organic sediments^{4,5}. These factors question the reliability of the Björkeröds Mosse data and thus the confidence with which the authors reject all previous results.

In contrast, I have described evidence for a Late Weichselian excursion from a single lithology of exceptionally uniform clay varves from Stårnö, Sweden². Studies of similar varves^{5,6} show that such clays were magnetised depositionally and if sedimentation occurred on a horizontal surface in the absence of strong water currents then the remanence is an accurate record of the ambient field. This is shown, for example at Stårnö, by the virtual absence of noise in varves which satisfy these criteria. The Revised Swedish Time Scale also provides a precise chronology within which to assemble the history of an excursion. At Stårnö the varve data were characterised by a reversal of declination and a shallowing, but not a reversal, of inclination while elsewhere in Sweden southerly declinations with

positive inclinations are a feature of younger varved clays (~8,000–7,600 BC) (ref. 5). As the Björkeröds Mosse core was unoriented, declination values are arbitrary and cannot be used to contradict these observations. Furthermore, according to Berglund⁷ it is impossible to correlate the varved clays in layer 1 with the Swedish Time Scale thus raising doubts concerning any direct age comparison between the Björkeröds Mosse data and results from dated varves.

Thompson and Berglund are correct in emphasising the need for caution when interpreting palaeomagnetic information from sediments, but their suggestion of using the repeatability of between-core directions as a major criterion for confirming the direction of the palaeofield could be misleading. Water movements in a basin are capable of producing large deflections⁸ which can be coherent at separate localities and a similar effect could arise through changing bedding errors⁹ as a basin infills. Thus observation of between-core coherence arising from these factors may mistakenly lead to a 'within-site reinforcement syndrome'. Thompson and Berglund's three-item criteria must be qualified according to individual circumstances and only a detailed consideration of all factors at each locality can decide the reliability of palaeofield directions. The Stårnö data survive as important evidence for a Late Weichselian excursion in Sweden which will achieve creditability as a world-wide phenomenon if palaeomagnetic results from sediments reveal global similarities, yet this is the objective which the authors seek to denounce.

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THOMPSON AND BERGLUND REPLY—Noël¹ assumes that precise palaeomagnetic data are an accurate record of the ancient geomagnetic field. We distinguish between precision and accuracy, and stress the importance of within-site repeatability as one requirement for the recognition of accurate palaeomagnetic recording of geomagnetic excursions.

Noël questions the reliability of our Björkeröds Mosse data² because of his qualitative assessment of the high variability of the sediments and scatter of palaeomagnetic directions. Inspection of the palaeochemical diagrams (Fig. 1 of ref. 3), rather than simply the column of layer numbers, shows that between ~13,000 and 11,000 BP the rate of sedimentation varied from only 50 to 100 mm per 100 yr, the sediment was never coarser than clay grade and that certain of the 'sedimentary units' alluded to by Noël are in fact transition zones. The mean direction of the oriented, cleaned, normally magnetised subsamples spanning the period ~13,000–11,000 BP is dec.=2°, inc.=66°, giving unit weight to each core, with an average 95% cone of confidence of radius of only 3.5° for each core section. We cannot agree with Noël's summary that these data are from sedimentologically complex cores with high noise content.

Noël stresses that "The Revised Swedish Time Scale also provides a precise chronology" for varved clays. Most Swedish Quaternary geologists are now convinced that there is no revised absolute time scale—based on varves—available for South Sweden. There seems to be a discrepancy of 600–800 yr between the varve years and the radiocarbon years south of the Central Swedish Moraines (B.B., in preparation). This is partly due to complications in the varve chronology, partly due to ¹⁴C variations in the atmosphere. Noël consequently uses a floating chronology which is less accurate than the biostratigraphic zones and transferred chronozones of the Björkeröds Mosse sections.

Noël misrepresents our conclusions. Correctly stated they are, "The Swedish late Weichselian reversal has become an example of Watkins's reinforcement syndrome" and "Furthermore, reversals or excursions of the geomagnetic field from other parts of the

world around this time should not be dated or correlated with the earlier Swedish results".

Finally, Noël incorrectly discusses our three minimum requirements as though they were absolute criteria. We reiterate that our criteria are to be regarded as minimum practical criteria for recognising geomagnetic excursions in recent sediments and that they represent only the first of several hurdles which a geomagnetic excursion must pass before gaining recognition as a global phenomenon.

Fairbridge⁴ has recently suggested the Late Weichselian geomagnetic 'reversal' is correlated with a short sharp cold fluctuation in climate. He discusses a mechanism by which climate could be controlled by geomagnetic changes. We would explain such a correlation by an opposite mechanism, namely that sudden climatic changes produce variable sediments which increase the probability of spurious palaeomagnetic data and hence apparent geomagnetic changes.

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¹ Noël, M. *Nature* 267, 180 (1977).

² Thompson, R. & Berglund, B. *Nature* 263, 490-491 (1976).

³ Berglund, B. E. & Malmér, N. *Geol. Förr. Stockh. Förrh.* 93, 575-589 (1971).

⁴ Fairbridge, R. W. *Nature* 265, 430 (1977).

Liquid nitrogen enhancement of partially annealed fission tracks in glass

PILIONE and Gold¹ described a means of revealing partially annealed fission tracks in glass by immersing the glass (NBS SRM 962)² into liquid nitrogen. According to their discussion it was possible to increase the total number of etchable tracks by increasing the immersion time of the glass in liquid nitrogen. Using this information we attempted to duplicate this work using the same NBS SRM 962 glass.

In a preliminary experiment, we immersed a piece of SRM 962 glass wafer into liquid nitrogen for approximately 8 h. Before the immersion the

glass had been heated to 230 °C for 1 h such that 57% of the tracks were annealed and then the glass was polished. This piece of glass and an unannealed piece, which had previously been broken from the same SRM 962 wafer, was etched in 16% HF at 23 °C for 75 s. Then the fission tracks in both pieces were counted and the track densities compared. The track counting statistics, however, were such that a definitive conclusion could not be drawn.

The experiment was repeated using several pieces of the SRM 962 glass and different continuous liquid nitrogen immersion times. At the end of each given immersion time, pieces of the glass were removed, allowed to reach room temperature, polished, and etched in HF as before. The results of this experiment are summarised in Table 1. We found no significant change with respect to the increased number of etchable tracks in the glass after its immersion in liquid nitrogen.

From our results we conclude that liquid nitrogen has no effect on the annealed tracks in glass. Subsequent discussions with Pilione and Gold have indicated that this effect was only observed when the glass had been polished before exposure to nitrogen. Therefore our conclusion is that the enhancement of partially annealed tracks is perhaps a surface phenomenon and has no effect on the interior matrix of the glass.

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² Carpenter, B. S. & Reimer, G. M. *NBS Spec. Publ.* 260, 49 (1974).

PILIONE AND GOLD REPLY—It is apparent from discussions between Pilione and Carpenter that different criteria are used for the identification of fission tracks in glass. But any 'track identification' difference should not affect the results if the density of 'artefacts' remained constant with immersion in liquid nitrogen, because the values reported were normalised in ratio form. We are investigating the effects of

chemical etchants on fission tracks and 'artefacts' in glass, in order to resolve this dilemma. We intend to report in the near future on recent experiments indicating the enhancement effect we noted earlier¹ is probably a surface rather than a volume phenomenon².

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Lack of correlation between tight junction morphology and permeability properties in developing choroid plexus

MOLLGARD *et al.*¹ report on morphological study of the sheep choroid plexus during foetal development. Excellent freeze-fracture replicas clearly show no difference in the ultrastructural features of the tight junctions in the choroid epithelium between 40- and 125-d foetuses. The authors reasonably agree that changes in tight junction strand number cannot account for alterations in permeability properties during development, since changes in strand number do not occur.

The reader unfamiliar with the blood-brain-cerebrospinal fluid system is likely to be left with the impression that these results disprove the existence of a correlation between tight junction morphology and permeability in the choroid epithelium itself and therefore in epithelia in general. This is not so. All the permeability studies²⁻⁴ referred to were made in intact animals or foetuses, in which transport of a solute between blood and cerebrospinal fluid can occur indirectly through the walls of the cerebral vessels and the interstitial fluid of brain as well as directly across the choroid epithelium. The observed developmental alterations in permeability with respect to cerebrospinal fluid are probably partly, and perhaps wholly, due to maturation of the cerebral vessels, that is, of the blood-brain barrier^{2,3}. Unless the authors or others can as clearly show for the cerebral endothelial cells of the sheep what has been so well demonstrated for the choroid epithelium, namely that there is no morphological change in the tight junctions of the blood-brain barrier from 40 d gesta-

Table 1 Effect of liquid nitrogen immersion on partially annealed fission tracks in glass

Glass condition		Track density* (tracks mm ⁻²)	Track retention
Not annealed		402.3	100%
Annealed, 230 °C for 1 h	No N ₂ treatment	231.4	57.5%
	1 d in N ₂	231.0	57.4%
	7 d in N ₂	228.2	56.7%
	30 d in N ₂	223.7	55.6%

*Counting statistics (1σ) is ±3%.

tional age to full development, no unequivocal conclusions can be made concerning structure and function in the tight junctions of the choroid plexus from the recorded observations.

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¹ Møllgård, K., Malinowska D. H. & Saunders, N. R. *Nature* 264, 293-294 (1976).

² Bradbury, M. W. B. et al. *J. Physiol., Lond.* 227, 591-619 (1972).

³ Evans, C. A. N., Reynolds, J. M., Reynolds, M. L., Saunders, N. R. & Segal, M. B. *J. Physiol., Lond.* 238, 371-386 (1974).

⁴ Dziegielewska, K. M. et al. *J. Physiol., Lond.* 258, 86P-87P (1976).

MØLLGÅRD *et al.* REPLY—We thank Dr Bradbury for raising an important aspect of the interpretation of our work which was insufficiently dealt with in our letter because of lack of space. In parallel with our studies of the choroid plexus and cerebrospinal fluid (CSF) we have also studied the ultrastructure of cerebral vessels and penetration of the same markers (non-electrolyte and plasma proteins) into developing brain. We have previously published results of ultrastructural studies of vessels in the immature brain¹. These results, although preliminary, suggested that interendothelial cell tight junctions develop very early in gestation. This has recently been confirmed (unpublished observations) in sheep brain as early as 30 d gestation. The strand number seems to be greater than in the choroid plexus at all ages so far examined. It is technically more difficult to obtain freeze-fracture replicas of cerebral endothelial tight junctions, so it is doubtful if it will be possible to provide evidence for endothelia on the scale already obtained for the epithelial cells of the choroid plexus. However, all endothelial cell junctions so far examined, either by freeze-fracture or by thin section, have had the appearance of well formed tight junctions.

Our permeability data (ref. 2 and unpublished) in 60-d sheep fetuses show that there is more restriction on penetration of protein from blood into brain than into CSF. This implies the presence of junctions which are functionally tight to protein. The data on foetal sheep electrolytes in CSF and plasma³, mentioned by Dr Bradbury, showed that CSF-plasma gradients were present for chloride and magnesium by 60 d gestation. This probably indicates the presence of both the appropriate ion pumps and a permeability barrier adequate to prevent loss of the gradients. Our non-electrolyte

data (ref. 4 and unpublished) suggest that inulin and sucrose penetrate into brain and CSF to a similar degree if the brain extracellular space is assumed to be equivalent to the chloride space. It may be, however, that these materials reach the brain in early development predominantly through the choroid plexus since this is disproportionately large in the immature fetus and the brain is poorly vascularised.

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³ Bradbury, M. W. B. et al. *J. Physiol., Lond.* 227, 591-610 (1972).

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Collagen microfibrils and cell attachment proteins

PEARLSTEIN¹, following Klebe² in the search for a cell attachment protein (CAP) (ref. 3) between mesenchymal cells and collagen, seems to disregard the lesson learnt from Jamieson's similar work on platelets⁴. They omitted to examine the microfibrillar structure of their reconstituted collagen substrata¹⁻⁴. Pearlstein¹ treats his collagen with 8 M urea to minimise 'non-specific' adhesion (see below). But he fails to note that urea inhibits fibrillogenesis⁵, or that collagen-binding both by platelets⁴ and fibroblasts⁶ depends crucially on microfibril aggregation, which also has a key role in the adhesion of BHK cells to a microfibril-forming polysaccharide substratum^{7,8}.

Moreover, the divalent ion requirements of CAP^{1,2} seem to conflict with those of other experiments^{9,10} which do not require CAP for adhesion to various forms of collagen. Rabinovitch and DeStefano showed that Mg or Mn by themselves promote adhesion of carcinoma cells to gelatin⁹, and I have confirmed this for BHK cells on adsorbed films of soluble collagen¹⁰.

Therefore, the fact that aggregation and divalent cations, respectively, can promote adhesion independently of

CAP calls for re-assessment of the "background" of "non-specific" adhesion (that is, in the absence of CAP) subtracted by Pearlstein¹ and Klebe^{2,3}. I suggest that their background adhesion, far from being an inconsiderable artefact, may actually represent a physiologically significant attachment of the mesenchymal cells to aggregated, microfibrillar parts of the reconstituted collagen substrata, under the influence of divalent cation alone; while their CAP-specific adhesion may be to a single-stranded (for example, urea-treated) form of collagen, not found in intact connective tissue. So far, unfortunately, CAP does not seem to have been tested on a physiologically intact form of collagen, such as omentum.

Hence the experiments on mesenchymal cells¹⁻³ should be repeated as those on platelets⁴ have been, that is, with confirmed microfibrils^{5,6}. Moreover, since there is evidence that various mesenchymal cells^{7,8,10-12} explore micro-architectural and mechanical aspects of their substrata, critical work with mesenchymal cells on collagen should also describe the size, spatial distribution and rigidity of microfibrillar particles.

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² Klebe, R. J. *Nature* 250, 248-251 (1974).

³ Kleinman, H. J., McGowan, E. B. & Klebe, R. J. *Biochem. biophys. Res. Commun.* 72, 426 (1976).

⁴ Jamieson, G. A., Urban, C. & Barber, A. J. *Nature* 234, 5 (1971).

⁵ Barnes, M. J., Gordon, J. L. & MacIntyre, D. E. *Biochem. J.* 160, 647-651 (1976).

⁶ Gordon, J. L. & Dingle, J. T. *J. Cell Sci.* 16, 157 (1974).

⁷ Maroudas, N. G. *Nature* 254, 695-696 (1975).

⁸ Maroudas, N. G. in *Supramolecular Structure: Cell Shape and Surface Architecture* (eds Henning, U. & Revel, J. P.) (Liss, New York, 1977).

⁹ Rabinovitch, M. & DeStefano, M. J. *J. Cell Biol.* 59, 165 (1973).

¹⁰ Maroudas, N. G., *J. cell. Physiol.* (in the press).

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¹² Dunn, G. A., and Heath, J. F., *Expl Cell Res.* 101, 1-14 (1976).

Stereospecificity and clinical potency of neuroleptics

ENNA *et al.*¹ have drawn attention to the potential, and some of the difficulties, of studies of the stereospecificity of neuroleptic drugs in elucidating the anti-schizophrenic action of these compounds. Although they have provided new data of considerable value we feel that they have obscured one issue. There is an increasing body of evidence on the stereospecificity of these drugs in *in vitro* assay systems and some evidence on their effects on animal behaviours, but as far as we are aware there are no systematic studies

of the stereospecificity of the anti-psychotic effect in man. Illnesses comparable with schizophrenia have not been identified in animals although some drug-induced models are available.

Enna *et al.* state that " α -flupenthixol and *cis*-thiothixene are much more potent clinically and in animals than their geometrical isomers β -flupenthixol and *trans*-thiothixene respectively. Similarly (+)-butaclamol is much more potent than its optical isomer (–)-butaclamol". These statements are supported by references which include data from animal behavioural experiments but no clinical findings. Therefore there seems to be a risk that the argument will be accepted that data obtained from an animal model of psychosis (for example amphetamine-induced abnormal behaviours) can provide definitive information concerning the nature of schizophrenia or its response to drugs. We suggest that such information can only be obtained from studies on patients with the disease, although this is difficult and time consuming.

If such studies can be designed to include not only a comparison of one isomer with another but of both with a placebo-treated group they can, however, in spite of the difficulties emphasised by Enna *et al.*, yield information beyond that relating to the *in vitro* receptor systems recently studied. If, for example, it can be shown that α -flupenthixol is clinically more active than the β -isomer and the latter is more active than placebo we may conclude on the basis of the data of Enna *et al.* not only that blockade of the dopamine, and possibly 5-HT, receptor may be relevant to the mechanism of action but that another action is also involved. If, on the other hand, the α -isomer is potent and the β -isomer is no more active than placebo, it seems safe to conclude that actions on those receptors on which the two isomers have been shown to be equally active (for example cholinergic, adrenergic and opiate receptors) are not relevant to the therapeutic effect.

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ENNA *et al.* REPLY—Crow *et al.*¹ rightly pointed out the importance of clinical studies in establishing for certain whether drugs, such as isomers of

Table 1 Neuroleptic stereoselectivity at α -noradrenergic receptor binding sites in rat brain

	Affinity for α -receptors	
	K_1 (nM)	Relative stereoselectivity
(+)-Butaclamol	35	70-fold
(–)-Butaclamol	2,500	
<i>cis</i> -Thiothixene	6.6	
<i>trans</i> -Thiothixene	150	23-fold
α -Flupenthixol	10	
β -Flupenthixol	36	3.6-fold

α -Receptors in membranes from whole rat brain were labelled by binding of the potent α -antagonist ³H-WB-4101 (2-([2', 6'-dimethoxy] phenoxyethylamino) methyl benzodioxan) subtracting as blank values binding in the presence of 100 μ M (–)-noradrenaline. Inhibition constants (K_1) were determined by measuring the effects of 3–4 concentrations of each drug in triplicate.

neuroleptics, possess anti-schizophrenic activity. It is commonly assumed that neuroleptics act therapeutically in schizophrenic patients by blocking dopamine receptors so that the isomer which fails to act on dopamine receptors would be anticipated to lack clinical effectiveness. This is only a presumption, which must be directly examined in clinical studies. If supposedly inactive isomers have therapeutic effect, one might assume that they exert part of their action through a system which does not display stereospecificity.

Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

The focus of our paper² was that some neuroleptics are stereospecific at 5-HT as well as dopamine receptors so that a stereospecific pharmacological action does not necessarily involve only dopamine synapses. Recently we have succeeded in identifying α -noradrenergic receptor binding in brain membranes using an agonist ³H-clonidine or antagonist ³H-WB-4101 (refs 3,4). Several neuroleptics have very high affinities for the α -noradrenergic receptor, often similar to their affinities for dopamine receptors⁵. But, the relative potencies of neuroleptics differ in competing for α receptors and dopamine receptors. Affinity for α receptors parallels closely pharmacological tests of α -noradrenergic blockade and tends to correlate with the ability of neuroleptics to elicit

sedation and orthostatic hypotension⁵. We have now found that neuroleptics display marked stereoselectivity at α -adrenergic receptor sites. The extent of stereoselectivity for the butaclamol isomers is as great at α -noradrenergic receptors as at dopamine receptors (Table 1). The potency of *cis*-thiothixene at α -noradrenergic receptors is similar to its potency at dopamine receptors, though the 23-fold stereoselectivity is somewhat less than that at dopamine receptors. The extent of stereoselectivity of flupenthixol isomers at α receptors is substantially less than at dopamine receptors. As some clinical improvement in psychiatric patients, including schizophrenics, could be associated with 'tranquilizing' effects secondary to α receptor blockade, these receptors and their stereoselective interactions with neuroleptics represent yet another neurotransmitter system which should be taken into account in explaining neuroleptic pharmacology.

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² Enna, S. J., Bennett, J. P., Burt, D. R., Creese, I. & Snyder, S. H. *Nature* 263, 338–341 (1976).

³ Greenberg, D. A., U'Prichard, D. C. & Snyder, S. H. *Life Sci.* 19, 69–76 (1976).

⁴ U'Prichard, D. C., Greenberg, D. A. & Snyder, S. H. *Molec. Pharmac.* (in the press).

⁵ Peroutka, S. J., U'Prichard, D. C., Greenberg, D. A. & Snyder, S. H. *Neuropharmacology* (in the press).

reviews

History of African crops

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Origins of African Plant Domestication. Edited by J. R. Harlan, J. M. de Wet and A. B. L. Stemler. Pp. xiii+498. (Mouton: The Hague and Paris; Aldine: Chicago, 1976.) Dfl.84.50; \$32.50.

THIS book contains the papers of the 58th Wenner-Gren Symposium on the origin of African plant domesticates, held at Burg Wartenstein in August, 1972. It opens with a series of three general papers, on the origins of domestication and of agriculture in Africa (Harlan, de Wet, Stemler), on causes (Pfeiffer) and on the problems of philosophy, technique and evidence (Higgs). Between them, they spell out the central question—at what periods, where, and why did African people begin deliberately to plant, tend and harvest some of the indigenous food and other useful plants of Africa; and how far, in doing these things, were they affected by influences from Asia? Over these topics controversy has smouldered for many years, alongside the more general endeavour to describe, more satisfactorily, the nature of the Neolithic transition and the emergence and spread of agriculture.

The climatic and ecological scene is set by van Zinderen Bakker. During the last glacial period, up to about 12,000 yr ago, Africa was dry, so that the Sahara spread as far south as 13°N (not S, as an oversight suggests). In a subsequent more or less warm and wet period, cattle, crocodiles and men could inhabit much of what is now desert, and the internal delta of the White Nile reached almost as far north as the modern junction of the Niles at Khartoum. This gave way about 4,500 yr ago to a cooler and drier period which may have driven Saharan man from an Eden of hunting, gathering and fishing into the drudgery of agriculture.

Seven archaeological and cultural/historical papers follow. Three are general (Clark, Shaw, P. E. L. Smith) and four more specialised (Munson, Flight, David, Wendorf and Schild). The evidence comes from a regrettably small number of sites, and there is not yet enough of it to tell a continuous or coherent story. Wheat and barley

from Asia were grown in Egypt more than 7,000 yr ago. It is easy to be convinced that indigenous *Pennisetum*, after being gathered for several thousand years, had given mankind a new crop, bulrush millet, by about 2,500 yr ago. Sorghum and cowpeas appear in West, East and Southern Africa in archaeological contexts, none of which is more than 1,500 yr old. From the Ethiopian plateau there is only one early record, of barley and chickpea, at about 2,500 yr ago.

The last seven papers are on botanical and agricultural topics. Purseglove presents a useful compendium on the crops domesticated in Africa, the history of exchanges between Africa and Asia, and the entry of American species. An essay by Harris on the origins of agriculture in West Africa is based too firmly for me on geographical and ecological 'first principles', but many will find in it, as its author hopes, useful hypotheses to be tested against observations, new and old. Scudder's brave reconstruction of prehistoric land-use in Zambia is illuminated by studies of modern agriculture in the same region. Then the botanists have the last words—on yams (Coursey) and African cereals: sorghum (two papers by de Wet, Harlan, Price and Stemler) and other species (by the late Professor R. Portères, a valuable English summary of over thirty years of devoted and scholarly labour). The two sorghum papers base a geographical history of the cultivated sorghums, starting with initial domestication in the eastern part of the sub-Saharan region, on a range of modern biosystematic and agricultural criteria, but in the absence of early archaeological records the timescale has to depend on the records of sorghum in India 3,000 yr ago.

This book is valuable because it assembles much of what is known, what is said to be known, and what is thought at the present time about the history of African crops. It is strongly suggested that the chief African cereals, and the domesticated yams, emerged without benefit of influences from outside Africa. But the timescales are tantalising: most if not all of the known history happened after agri-

culture had been established in the Nile Valley, and long after it was the basis of a way of life only 400 km away at Jericho and of course further north and east. It is difficult to imagine that the news did not get around. Man, including African man, is far too mobile for that. One wonders whether African oral tradition, difficult though it may be to date, has not a contribution to make here.

It must be important to learn more about human movement in antiquity: the apparently ancient navigation of the Indian Ocean from China to Ceylon, the Gulf, Suez and East Africa, the links across the Red Sea between the similar agricultures of the highlands of Yemen and of Ethiopia, the east-west traffic through what are now the Sahelian and savanna zones from the Blue Nile region (where every *jebel* seems to have been a workshop in days gone by) to the Atlantic; the north-south traffic across what is now the desert from the Mediterranean and the Nile, along the routes—like the *Darb al Arba'in*, the road of forty days—to Kordofan, Darfur, the Tibesti and West Africa (which some suggest brought the builders of stone circles to Senegal and the Gambia, although we know not when).

Then I am tantalised by querns and mortars, the two distinct means by which African people grind grain. The quern, an ancient device in Asia, seems to have spread in Africa before agriculture; the mortar seems to be African. Are the crops and the means for preparing them historically related, and if so, how?

Finally a question about vegetation. Chevalier divided the different sorts of vegetation he saw in West Africa into the forest, savannah and other zones. These correspond broadly with current climates, and many people seem to regard them as 'natural' biogeographical divisions. Yet without exception they are artefacts of agricultural man. What did West Africa look like in the wetter and warmer period 6,000 yr ago, when farming was at most in its infancy? □

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Distinguished Arctic explorer

Sir John Richardson: Arctic Explorer, Natural Historian, Naval Surgeon. By R. E. Johnson. Pp. xii+209+33 plates. (Taylor and Francis: London, 1976.) £15.

It is remarkable that this should be the first attempt at a full-length biography of Sir John Richardson since the account by his nephew, John McIlraith, in 1868.

It comes as no surprise to learn that the man who distinguished himself in Arctic exploration, and natural history and introduced reforms in medical practice, was a precocious youth. Apprenticed to a surgeon at the age of thirteen, Richardson began his medical studies at the University of Edinburgh the following year. A little before his twentieth birthday he joined the Royal Navy as an assistant surgeon.

The direction of his life was decided when he was made surgeon and naturalist on an overland expedition to the Canadian Arctic Ocean under Lieutenant John Franklin in 1818. During the next three years this expedition covered 5,500 miles in appalling conditions, losing eleven men, one of whom Richardson was forced to shoot in self-defence. Richardson joined Franklin again as surgeon and naturalist on his second Arctic expedition in 1825, and when Franklin failed to return from his third expedition in 1845, Richardson at the age of sixty-one set out for the Arctic once more in a vain attempt to find his friend.



Sir John Richardson

Mott Collection, Cambridge

Richardson's scientific career began with his zoological and botanical appendices to Franklin's and Parry's accounts of their Arctic expeditions. Sir W. J. Hooker's *Flora Boreali-Americana* (1840) was based principally on the plant collections of Richardson and his very able assistant, Thomas Drummond. His scientific reputation became firmly established through his contributions to the four-volume *Fauna Boreali-Americana* (1829-1837), a pioneer work in Arctic biology.

During his time as physician at the Royal Naval Hospital, Gosport, he created a museum of comparative anatomy and taxonomy which was consulted by scientists as eminent as

Darwin, Lyell, Hooker and Gray. Richardson was a prodigious worker, as the complete bibliography of his books and periodical articles in this book testifies.

Very little seems to have escaped the author's diligent searching for every scrap of information about Richardson. Even the translated text of Richardson's thesis for his doctorate in medicine, *De Febre Flava* (1816), has been included. Research workers in this field will find his list of the main collections of Richardson's letters useful. One of the appendices lists all the known portraits of Richardson followed by the species of animals named by or after him, but through some oversight the plants have been omitted.

In a work that is so full of facts some errors are inevitable: Richardson was elected a Fellow of the Royal Society in 1825, not 1835 (p65); J. E. Gray retired as Keeper of the Department of Zoology at the British Museum in 1874, not 1872 (p114); Sir W. J. Hooker was appointed Director of Kew Gardens in 1841, not 1847 (p114).

Dr R. E. Johnson has methodically assembled all the data necessary for a definitive life of Sir John Richardson. But Richardson does not come alive through his pen, and there is no real assessment of a naturalist who was also one of the outstanding ichthyologists of the nineteenth century.

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Process of language understanding

Computational Semantics: An Introduction to Artificial Intelligence and Natural Language Comprehension. Edited by E. Charniak and Y. Wilks. Pp. vii+294. (North-Holland: Amsterdam, New York and Oxford, 1976.)

"COMPUTATIONAL SEMANTICS", say Charniak and Wilks in the preface to their book, "is not so much a new subject as a new way of looking at old questions—those concerning meaning, language and understanding. It is based on the assumption that a good way to explicate such difficult notions is to work toward the programming of an automaton, or digital computer, so that it could be said to understand language." This assumption characterises much recent research in Artificial Intelligence (AI), and it is the AI viewpoint which is strongly put forward by this book.

To demonstrate the significance of this viewpoint, Charniak, Wilks and the other contributors to this volume have placed computational semantics within the context of related language disciplines, providing a broad overview of linguistics, the psychology of language and memory, the philosophy of language, AI approaches to language comprehension, and fundamentals of computer programming. In bringing together all of these cognitive science topics in a tutorial format, the editors have taken an important step towards interdisciplinary consciousness raising. Such an ambitious step is not without flaws, of course—the most serious being the use of heavy attacks on straw men within generative semantics and psychology in order to glorify AI approaches to language comprehension. The drawing of these 'battle lines' detracts from an otherwise noble cause.

The book stands strongly on its positive treatment of tother topics—for instance, it provides an excellent survey of AI language comprehension programs, particularly in the second of Charniak's

two chapters on inferencing and the second of Wilks' two chapters on parsing. Both of these chapters provide clear accounts of recent AI research on language understanding, and suggest some new dimensions along which various AI programs may be compared.

Students interested in theories of language, meaning, and understanding would be well advised to read this book, and to develop a familiarity with all of the areas it covers. Psychologists, linguists and philosophers will no doubt find fault with the sections dealing with their own specialities. If they keep an eye out for straw men, however, they will find that the other sections of the book provide a fine background to some of the most exciting contemporary research into the processes of language understanding.

Marc Eisenstadt

Marc Eisenstadt, formerly a Research Fellow at the Department of Artificial Intelligence, University of Edinburgh, is now Lecturer in Psychology at The Open University, Milton Keynes, UK.

Major histocompatibility complex

Histocompatibility. By George D. Snell, Jean Dausset and Stanley Nathenson. Pp. xiv+401. (Academic: New York, San Francisco and London, 1976.) \$29.50; £18.

THIS is an excellent book for seasoned professionals and advanced students with a substantial background in mammalian genetics and immunology. Emphasis is on the much studied, major histocompatibility complex (MHC) of mice (*H-2*) and men (*HLA*). It is difficult to find fault with this masterfully written treatise. The coverage is thorough and well-documented. The subject index is admirably detailed. The list of abbreviations given at the outset is helpful in coping with inevitable acronyms. Because so much of the current literature deals with the findings of the past few years, it is also refreshing to encounter each theme in historical perspective. One only wishes the scope extended to non-mammalian vertebrates and invertebrates, but this is newer territory awaiting exploration in depth. This superbly comprehensive volume will be the definitive reference for histocompatibility characteristics of mice and men for years to come. If progress continues at the present rate, however, an updated edition will surely be desired about 1980. The principles so nicely explained in this book should of course remain valid, but the details will surely become more complex.

The first eight chapters take the reader progressively from essential principles of transplantation immunogenetics through allogeneic polymorphism, *H-2* complex loci, and immune response (*Ir*) genes in mice. In chapters 9, 10 and 12, the serology, genetics, and disease associations of the *HLA* complex in man are described in a cogent manner. Chapter 11 deals comparatively with the biochemistry of *H-2* and *HLA* alloantigens. The predominant role played by the MHC in cell interactions, especially lymphocyte interactions such as mixed lymphocyte reactions, cell-mediated lysis, T-B lymphocyte collaboration, and hybrid resistance in mice is admirably presented, especially in chapter 8.

At any given time, every field has a prevailing dogma and histocompatibility or transplantation immunogenetics is no exception. Although emphasis is on the MHC as the only gene complex imposing a "very strong barrier to transplants", the roles of other *H-* or *Ir* genes are trenchantly considered in chapters 3, 7, and 13, the final chapter. Even recipients of *HLA*-identical organ transplants will usually reject such allografts promptly in the absence of drug immunosup-

pression; immunodeficient recipients of allogeneic bone marrow regularly succumb to fatal graft-versus-host disease despite identity for all four *HLA* loci. Other *H* systems then may often have 'major' impact. Moreover, as the authors aver in several chapters, slight preimmunisation with donor-type alloantigens may convert a weak non-MHC barrier into a strong one. Uniqueness of the MHC, if indeed each species has only one such *main system* (book italics), apparently resides in the contiguity of the constituent loci and their interaction. Many of the constituent *H-2* loci taken singly in tests between recombinant congenic lines have effects similar to moderate non-*H-2* loci. In light of other recent evidence, much of the mammalian genome may be organised into large functional units with relevant developmental and regulatory information associated with component structural genes.

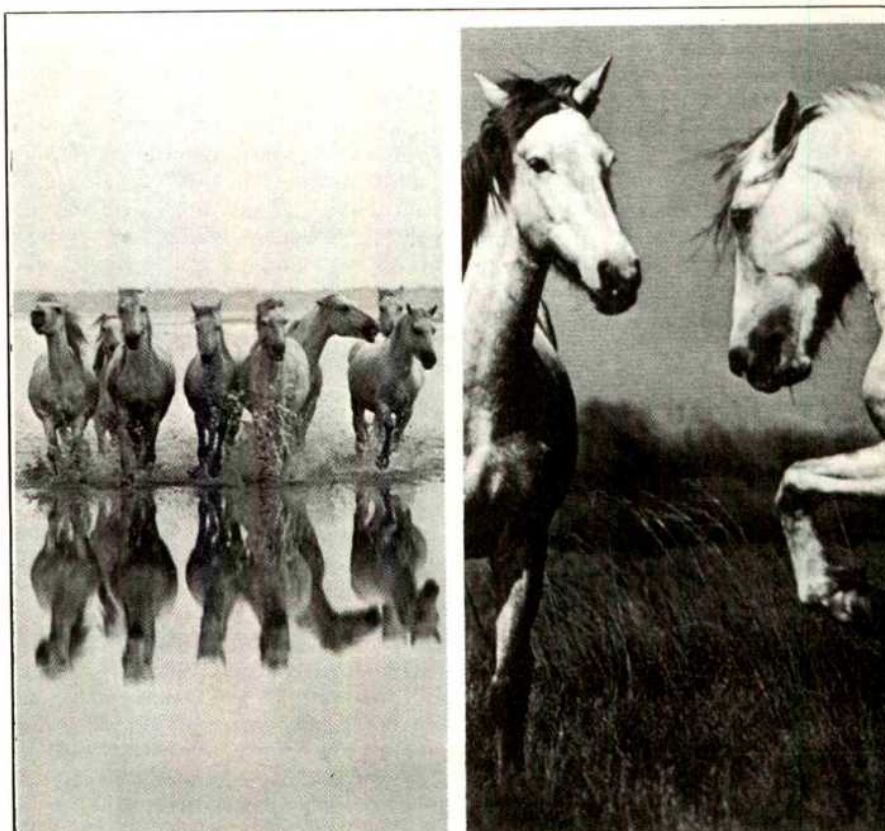
Snell, Dausset and Nathenson deal perceptively with discordant findings. For example, divergent data concerning the genetic 'laws' of transplantation, sources of specific immune blocking, and the chemistry of MHC antigens are evaluated in a commendably evenhanded manner. Moreover, they suggest further experiments to resolve ambiguities. An understanding of sequential *HLA* gene-antigen relationships in man has now become difficult for non-specialists. An illustrative diagram proceeding from the chromo-

some No. 6 loci→ alleles and antigens→ haplotypes→ phenotype would have been useful in chapter 9 to epitomise the immunogenetics of the *HLA* system.

Chapter 7 deals concisely with immune response genes and probable mechanisms of *Ir* gene action in mice. This theme is pursued again in man in an incisive chapter 12 on histocompatibility genes and disease—especially associations between *HLA* and disease susceptibilities. The authors favour the hypothesis that *Ir* and metabolic genes closely linked to the *HLA* genes are actually responsible for susceptibility or resistance. Although acknowledging the undoubted relevance of the *HLA* complex to medical genetics, the reader should retain reservations toward the 'MHC controls everything' viewpoint by remembering that many known *Ir* genes are not linked to the MHC; that only a few human diseases (all non-neoplastic) reveal a strong correlation with particular *HLA* specificities; and that animals below the phylogenetic level of advanced amphibians or bony fishes cope quite well immunologically without having evolved a mammalian-type MHC. Altogether, this volume is an outstanding contribution to the field of transplantation genetics and immunobiology.

W. H. Hildemann

W. H. Hildemann is Professor of Immunogenetics and Dental Research Institute Director at the University of California, Los Angeles, California.



Illustrations taken from *Horses of the Camargue*. Photographs and text by Hans Silvester. Preface by Konrad Lorenz. Pp. 23+70 pages coloured photographs. (Phaidon: Oxford, 1976.) £12.50.

Marine pollution handbook

Marine Pollution. Edited by R. Johnston. Pp. 729. (Academic: London and New York, 1977.) £23.40; \$51.

MARINE pollution texts are being published with almost monotonous regularity, and the subject is at best duplicated, but more often fragmentary and of poor perspective. The field is a complex one and calls for a multi-disciplinary approach, and it is not without significance therefore that Dr Johnston has chosen to produce a book by persuading colleagues to write chapters on various specialised aspects.

There are eight contributors to a volume of over 700 pages, with material organised into four principal sections. The first, by the editor, provides a broad brush background to the marine pollution scene, including aspects of fisheries biology and a brief review of the major categories of pollutants, their principal pathways in the marine environment and their potential effects. The second concerns a number of topics of importance to an understanding of the pollution problem, including material on dispersion in estuaries and coastal waters, on heavy metal contamination in the sea, on sewage pollution, on oil pollution and on the impact of pollution on seabirds. The third section is devoted to a review of the problems of measuring biological response; a very intractable problem in terms of the natural environment and one only showing signs of being amenable to treatment in terms of human health. The fourth and final section deals with the legal aspects of marine pollution regulation.

Marine pollution is a very emotive subject, and quite opposing views as to its significance are genuinely held by scientists of all disciplines engaged in oceanographic science. It would be surprising, therefore, if any reviewer could wholly agree with conclusions of one contributor, let alone eight. Nevertheless the book is remarkable in its attempt, particularly in the editor's first chapter, to provide an unbiased view of the subject; and the chapters by Bryan on metals and Topping on sewage are admirable and balanced treatments of their subjects, although Bryan's might have benefited from a slightly more quantitative treatment of the dynamics of metal uptake and loss in organisms.

Cowell's chapter on oil covers much familiar ground but is well presented and readable, although unfortunately marred at the end by an ambiguous statement according significance to mercury pollution. The persistence

of mercury could not conceivably create a global pollution problem since manmade input would have to be maintained at present rates for periods commensurate with the residence time of mercury in the oceans, say 10^4 yr, before a significant increase in the worldwide concentrations of mercury in the oceans could be achieved. It is therefore misleading to state that persistence can necessarily be equated with global contamination, although it may be in surface waters if the pollutant is distributed by way of the atmosphere, as in the case of DDT.

The chapter by Bourne on seabirds and pollution makes interesting reading, and he rightly points out that vulnerability of birds at the end of marine food chains, and the conspicuous way in which dead animals are seen on the strand lines of beaches, often provide the earliest indication of unacceptable situations arising at sea.

Muscular exercise

Biomechanics and Energetics of Muscular Exercise. By Rodolfo Margaria. Pp. x+146. (Clarendon: Oxford; Oxford University: London, 1976.) £7.

It is a pleasure to read a book by an expert expounding his life's work, an *auto-festschrift* as it were. Professor Margaria treats the reader to a feast cooked by a master chef, beginning with an historical note in which he himself appears as making a crucial contribution to the lactic acid-oxygen debt controversy. He leads us, by way of his own and his colleagues' work, through the intricacies of the sources of energy for muscular contraction, quantifying the contributions made by oxidation, glycolysis and phosphagen cleavage (the lactic acid and alactic oxygen debt). There follows a very short and somewhat cryptic chapter on some of the cardiorespiratory changes in exercise. Here is the only serious attempt within the book to use SI units; the style and content make one suspect another and less thoughtful hand. The third and final section is entitled "The biomechanics of human locomotion", in which there is a very clear description of how mechanical work may be measured and the interesting conclusions that may be drawn. The author holds out hope for the non-athletic that they too will be able to run at world (Earth) record speeds when the Olympics are held on the Moon.

The book reflects the character of the man; every now and then the

These, of course, are not always related to pollution.

The final chapter by Moore on legal aspects is a most useful review of the subject. Particularly welcome is the clear expression of the view that the function of marine pollution control is not to prohibit entirely the introduction of wastes to the sea, and that indeed the ability of the sea to absorb and assimilate waste is one of its most valuable resources: rather that marine pollution is part of the conflict between the need to release some contaminating materials to the sea and the desire to maximise the use of the marine environment as a source of food and for recreation and transport.

Alan Preston

Alan Preston is Deputy-Director of Fisheries Research in the Ministry of Agriculture, Fisheries and Food based at Lowestoft Laboratories, and Chairman of the UK Marine Pollution Monitoring Management Group.

complex and erudite arguments are interspersed with friendly pieces of practical advice, inviting the reader to relax his mental grip just for a moment. Nevertheless, the book is not for the faint-hearted, the skimmers or the dippers; it is closely argued and requires dedicated concentration. The mathematics, of which there is a good deal, is helpful and yet simple to follow; no-one need fear the book on that account. The author pleads for the adoption of his findings in everyday life—this unfortunately is the *cri de coeur* of many human physiologists! He spoils the case a little, however, by presuming his own degree of expertise in others; how many, for example, would describe his test for maximum anaerobic power as simple? In a world in which rapid communication is producing a tendency to dull uniformity it is interesting to note that schools of thought may still differ on the most practical matters—in Milan the stepping stool reigns supreme for almost the same stated reasons that the bicycle ergometer holds sway in Sweden.

To whom then would this book be useful? Most definitely for the serious and advanced student, but it cannot be unreservedly recommended as a standard text. Although one may overlook the occasional slipshod editing of text and figures, the use of the old units (calories and kilogram-metres), with never a joule or a watt, panders to middle age, prolongs our difficulties and introduces unnecessary confusion for those nearer the starting line.

Rainer Goldsmith

Rainer Goldsmith is Professor of Physiology in the University of London, UK.

obituary

D. H. Menzel

PROFESSOR Donald Howard Menzel, past director of Harvard Observatory, who died in Boston, Mass. at the age of 75 years on December 14, 1976, was born on April 11, 1901 in Florence, Colorado, and educated at the Universities of Denver and Princeton where he received his doctorate in 1924. After several junior appointments of short duration he joined, in 1932, the staff of the Harvard University which remained his academic home until his retirement in 1971. Within his lifetime he took an active part in the transformation of the American scientific scene from its grass-roots stage to the "big science" which it has become—for better or worse—today.

When Menzel left Princeton in 1924, he was a young theoretical astrophysicist—of whom there were not many in the United States at that time—bent on applications of atomic physics to astrophysical situations, and his first interests centred on the physical processes in gaseous nebulae. In collaboration with several students of his who have since attained a renown of their own (and among whom L. H. Aller and J. G. Baker should particularly be mentioned) throughout the 1930s Menzel made important contributions to the subject which are still well-remembered today.

Another field to which Menzel then turned his professional attention, and to which he remained faithful for a major part of his life, was the Sun. The excitement which Bernard Lyot created in the late 1930s with his observations at Pic-du-Midi of the solar corona outside total eclipses, and his films of the activity of solar prominences, is still a vivid memory among the survivors of that heroic epoch. Menzel was the first to transplant these techniques across the Atlantic, and his efforts led to the foundation in 1939 of the High-Altitude Observatory in the Colorado Rockies near Climax, Colorado, at an altitude of over eleven thousand feet—first as an offshoot of Harvard, and later as an independent institution, which only recently closed its doors because of increasing pollution of its environment (caused by nearby mining activities).

Unlike Lyot, who was a genius at improvisation and preferred to perform experiments with his own hands (to the extent of carrying the crucial parts of his coronagraph to the top of Pic-du-



Midi on his back!), Menzel was a theoretician of managerial type, who worked through other people's hands. His field executive in the Climax operation was mainly Walter Orr Roberts, another student of Menzel's who retired only recently from the directorship of the National Centre for Atmospheric Research in Boulder, of which the High-Altitude Observatory eventually became a part.

Then war came (in which Menzel served his country in the uniform of a reserve naval officer) and with it changes in academic and scientific life which were fully reflected also in Menzel's subsequent career. Like many others, after 1945 Menzel never returned properly to resume the career of a scholar, and his activities veered increasingly towards those of an administrator and promoter of science. These latter abilities, which were at a premium during the years of post-war inflation, eventually led to Menzel's appointment in 1954 as Director of the (then) Harvard College Observatory—a position in which he succeeded Harlow Shapley and which he held for the next 12 years.

His illustrious predecessor possessed, among his many talents, an uncanny gift (perfected during his apprenticeship with G. E. Hale) to extract money for astronomy from rich Americans, with which to augment his observatory's current income. In postwar years this goal proved doubly desirable, but no longer attainable in the old ways; for fewer Carnegies were left in the world (income tax saw to that) and, instead, most available funds were to be found in the hands of the Federal Government in Washington. As a result,

Shapley's successor had to seek additional support for his institution, no longer by waiting on the rich, but by lobbying in the "corridors of power" in Washington for grants or contracts from different branches of the Government through their many agencies.

This was an ominous turn of events for the further development of American science; for while rich Americans were (sometimes) willing to make gifts for a good cause with no strings attached and very little paperwork, the new dispensers of Government largesse knew exactly what they wanted and saw to it that they got it. As a result, the Harvard Observatory during Menzel's regime got often involved in work in new fields, such as geophysics, in which it had neither tradition nor sufficient expertise. At times, the Observatory seemed to differ only in location, but not in spirit, from the many research laboratories which sprang up during the "roaring sixties" along Route 128 around Boston as offshoots of Harvard or M.I.T. to serve the customer rather than science—at a time when men were heading for the Moon, and when nothing seemed impossible on Earth.

It is remarkable that, in this atmosphere and with so many administrative preoccupations, Menzel found also time to write or edit a considerable number of books (by himself, or in collaboration with others), ranging from collections of mathematical formulae to polemics about UFOs. Some, like his popular book on the Sun, enjoyed a well-earned popularity in their time, but none seems to have left any deeper mark on contemporary science.

Needless to say, a life so busy has its drawbacks for institutions as well as the individuals directing them, and Menzel was no exception. His health suffered a serious setback in 1965 from which he never fully recovered. He retired from directorship in 1966, and although he continued to teach students until 1971, as a scientist Menzel was essentially on the side-lines—occupying his mind with problems of such gravity as lunar nomenclature, and leaving it to his successors to pick up the more important pieces of the game. Death claimed him last December at the age of 75 years, after a prolonged illness; he was survived by his wife (née Florence Kreager), two daughters and six grandchildren.

Zdeněk Kopal

announcements

Meetings

16 May, **Risky ways to safety—Technological Assessment and Public Benefit**, London (Council for Science and Safety, 3/4 St Andrew's Hill, London).

19 May, **Molecular Beams in Chemistry**, London (S. Langer, The Chemical Society, Burlington House, London W1).

11–15 June, **Annual Meeting of the Society for Economic Botany**, Coral Gables, Florida (G. J. Anderson, Society for Economic Botany, Biological Sciences U43, University of Connecticut, Storrs, Conn. 06268).

31 August–2 September, Cambridge, UK, **2nd International Conference on Drag Reduction** (Secretary, 2nd Drag Reduction Conference, BHRA Fluid Engineering, Cranfield, Bedford, UK).

4–8 September, **Low Energy Ion Beams**, Salford (The Institute of Physics, 47 Belgrave Square, London SW1).

5–8 September, **Acetylenes, Allenes and Cumulenes**, Nottingham (J. F. Gibson, The Chemical Society, Burlington House, London W1).

5–9 September, **24th International Field Emission Symposium**, Oxford (G. Smith, Metallurgy and Science of Materials, Oxford University, UK).

5–9 September, **8th L. H. Gray Conference-Hypoxic Cell Sensitisers in Radiobiology and Radiotherapy**, Cambridge, UK (G. E. Adams, CRC Gray Laboratory, Mount Vernon Hospital, Northwood, Middlesex).

5–10 September, **VIIIth International Congress—International Union For the Study of Social Insects**, Wageningen, The Netherlands (International Agricultural Centre, Lawickse Allee 11, P.O. Box 88, Wageningen, The Netherlands).

5–10 September, **Biology of Connective Tissue**, Uppsala, Sweden (T. C. Laurent, Institute of Medical and Physiological Chemistry, University of Uppsala, Sweden).

6–9 September, **Plant Cell and Tissue Culture: Principles and Applications**, Columbus, Ohio (4th Annual Colloquium, College of Biological Sciences, 484 W. 12th Avenue, The Ohio State University, Columbus, Ohio 43210).

10 September, **5th European Colloquium of Geochronology, Cosmochronology, and Isotope Geology**, Pisa (G. Bonadonna, C.N.R.-Laboratorio di Geochronologia, via Cardinale Maffi, 36, 56100 Pisa, Italy).

11–16 September, **11th World Congress of Neurology**, Amsterdam (F. H. Lopes da Silva, Institute of Medical Physics, Da Costakade 45, P.O. Box 5011, Utrecht, The Netherlands).

Person to Person

Exchange furnished family house in Oxford for similar in Cape Town. Available July–September 1977. Contact Dr Donald Fraser, Department of Geology, Oxford University, England.

Exchange furnished 17th century thatched cottage 4 miles from Cambridge for house near Stanford University Medical Center, California, from February 1978 to February 1979. Car exchange also. Contact Karol Sikora, MRC Laboratory of Molecular Biology, Hills Road, Cambridge, England.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

Reports and Publications

Other countries—March

United States Department of the Interior: Geological Survey. Bulletin 1427: Surficial Geologic History of the Canyon Village Quadrangle, Yellowstone National Park, Wyoming, for use with Map 1-652. By Gerald M. Richmond. Pp. iii+35. Professional Paper 957: Lead in the Environment. Edited by T. G. Lovering. Pp. iv+90. (Washington, DC: US Government Printing Office, 1976.) [83]

Of Politics and Drug Regulation. By G. Frederick Roll. Pp. 30. (Rochester, New York: The Center for the Study of Drug Development, University of Rochester Medical Center, School of Medicine and Dentistry, 1977.) [103]

United States Department of the Interior: Geological Survey. Water-Supply Paper 2043: Water in Carbonate Rocks of the Madison Group in South-eastern Montana—a Preliminary Evaluation. By W. R. Miller. Pp. v+51+2 plates. (Washington, DC: US Government Printing Office, 1976.) [113]

American Museum Novitates. No. 2613: On the Jaw Musculature and Relationships of *Petrodromus tetradactylus* (Mammalia, Macroscelidea). By Ronn W. Coldiron. Pp. 1–12. (New York: The American Museum of Natural History, 1977.) [113]

CERN—European Organization for Nuclear Research. CERN 76-24: Proceedings of the 1976 CERN School of Computing. La Grande Motte, France, 12–25 September 1976. Pp. viii+283. (Geneva: CERN, 1976.) [113]

Commission of the European Communities. Nuclear Science and Technology. Removal of Actinides from High Activity Wastes by Solvent Extraction: Outline of the Research Work at Ispra J.R.C. Laboratories. By F. Mannone. Pp. 41. (Luxembourg: Office for Official Publications of the European Communities, 1976.) FB 150; £1.85. [143]

The Polychaete Worms: Definitions and Keys to the Orders, Families and Genera. By K. Fauchald. (Science Series No. 28.) Pp.188. (Los Angeles: Natural History Museum of Los Angeles County, 1977. Published in conjunction with The Allan Hancock Found-

ation, University of Southern California.) \$6. [143]

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Protein Chemistry, 1975/1976. Pp. 39. (Melbourne: CSIRO, 1977.) [143]

Report on a Survey of the Membership of the American Astronomical Society Concerning the UFO Problem. By P. A. Sturrock. (SUIPR Report No. 681.) Pp. 202. (Stanford, California: Institute for Plasma Research, Stanford University, 1977.) [143]

CERN—European Organization for Nuclear Research. Descriptive Brochure. Pp. 20. (Geneva: CERN, 1977.) [143]

United States Department of the Interior: Geological Survey. Professional Paper 981: The Rinconada and Related Faults in the Southern Coast Ranges, California, and Their Tectonic Significance. By Thomas W. Dibblee, Jr. Pp. iv+55. (Washington, DC: US Government Printing Office, 1976.) [153]

Lei da Causalidade Universal: Teses e Hipoteses. By Waldeck Sampaio. Pp. 48. (Rio de Janeiro: Tip. Pap. Jofeleite, Ltda., Rua Santo Cristo 307, 1976.) [173]

Indian Forest Records—Silviculture. (New Series). Vol. XIII, No. 1: Population Dynamics of Forest Stands. By S. K. Seth. Pp. 179. (Delhi: Controller of Publications, 1974.) Rs. 11.50; £1.35; \$4.14. [173]

CERN—European Organization for Nuclear Research. CERN 77-03: Low-Temperature Irradiation Effects on Materials and Components for Superconducting Magnets for High-Energy Physics Applications. Edited by M. Van de Voorde. Pp. vi+69. (Geneva: CERN, 1976.) [173]

Smithsonian Contributions to Zoology. No. 243: Pseudanthessiid Copepods (Cyclopoida) Associated with Crinoids and Echinoids (Echinodermata) in the Tropical Western Pacific Ocean. By Arthur G. Humes. Pp. iii+43. (Washington, DC: Smithsonian Institution Press, 1977. For sale by US Government Printing Office.) [173]

United States Department of the Interior: Geological Survey. Professional Paper 759-B: Physiographic Subdivisions of the Chirikov Basin, Northern Bering Sea. By D. M. Hopkins, C. H. Nelson, R. B. Perry and Tau Rho Alpha. Pp. 7+3 plates. (Washington, DC: US Government Printing Office, 1976.) [173]

United States Department of the Interior: Geological Survey. Professional Paper 959-H: Alluvial Ilmenite Placer Deposits, Central Virginia. By J. P. Minard, E. R. Force and G. W. Hayes. Pp. vi+15+plate 1. (Washington, DC: US Government Printing Office, 1976.) [183]

Innovation in Science Education—World Wide. By Albert V. Baez. Pp. 249. (Paris: The Unesco Press, 1976.) [223]

World Health Organization. Report of the Regional Director, July 1975 to June 1976. Pp. 102. (Copenhagen: WHO Regional Office for Europe, 1976.) [233]

Publications of the Dominion Astrophysical Observatory, Victoria, BC. Vol. XV, No. 1: Photoelectric Measures of Variable Stars Observed at Mt. Kobau (1970–73). By Graham Hill, R. W. Hilditch and E. L. Pfannenschmidt. Pp. 35. (Victoria, BC: Dominion Astrophysical Observatory, 1976.) [233]

Republic of South Africa: Department of Industries, Sea Fisheries Branch Investigational Reports. No. 111: Comparison of the Food and the Filtering Mechanism of Pilchard *Sardinops ocellata* and Anchovy *Engraulis capensis* Off South West Africa, 1971–1972. By D. P. F. King and P. R. Macleod. Pp. 29. No. 112: Exploratory Fishing for and Growth of Scallop *Pecten sulcatus* Off the Cape South Coast. By G. De Villiers. Pp. 23. (Cape Town: Department of Industries, Sea Fisheries Branch, 1976.) [243]

United States Department of the Interior: Geological Survey. Professional Paper 902: Precambrian Geology of the United States; An explanatory Text to Accompany the Geologic Map of the United States. By Philip B. King. Pp. iv+85. Professional Paper 965: The Relation of Geology to Stress Changes Caused by Underground Excavation in Crystalline Rocks at Idaho Springs, Colorado. By Fitzhugh T. Lee, John F. Abel, Jr., and Thomas C. Nichols. Jr. Pp. iv+47+plate 1. Professional Paper 973: The Roberts Mountains Formation, a Regional Stratigraphic Study with Emphasis on Rugose Coral Distribution. By Charles W. Merriam and Edwin H. McKee. Pp. iv+51+plates 1–12. Professional Paper 985: Biostratigraphy and Regional Relations of the Mississippian Leadville Limestone in the San Juan Mountains, Southwestern Colorado. By Augustus K. Armstrong and Bernard L. Mamet. Pp. iii+25+plates 1 and 2. Professional Paper 1000: Geological Survey Research 1976. Pp. xi+414. (Washington, DC: US Government Printing Office, 1976.) [243]

Australia: Commonwealth Scientific and Industrial Research Organization. Land Research Series. No. 38: Lands of the Alligator Rivers Area, Northern Territory. Comprising papers by R. Story, R. W. Galloway, J. R. McAlpine, J. M. Aldrick and M. A. J. Williams. Pp. 171+3 maps. (Melbourne: CSIRO, 1976.) [253]

Smithsonian Contributions to Zoology. No. 219: Phylogeny, Ontogeny, and Morphology of Living and Fossil Thaumatocephalidae (Myodocopa: Ostracoda). By Louis S. Kornicker and I. G. Sohn. Pp. iv+124. (Washington, DC: Smithsonian Institution Press, 1976. For sale by US Government Printing Office.) [253]

nature

19 May 1977

No error-free society

NOTHING ventured, nothing gained—the calculus of risk and the trade-off between benefit and damage is much in the news at present. This is partly due to the growing public interest in decision-making in the nuclear industry, but some of the credit in Britain must go to the Council for Science and Society (CSS) which has had a study group looking at the question of acceptable risk and which this week convened a public meeting on the subject.

There is something almost irrational in our variable attitudes to risk-taking. The hazards of cigarette smoking are well enough known, are not by any means negligible, often involve others quite involuntarily in a long period of distress and yet are clearly deemed acceptable by a large section of the public. The same might be said of combining drinking with driving. And yet at the other end of the scale those same inveterate risk-takers may show profound shock on hearing of ten people being killed in a coach crash or may express the strongest opposition to the building of a nuclear power station with a claimed minute chance of having a serious accident. We may live in fear of a major accident in which a thousand might die even though, as Reg Farmer pointed out last week (page 93), such an event would barely affect the accidental death rate. It somehow seems that the idea of one or two people being responsible for many deaths strikes a very profound note in the public conscience. Desirable as this is, it inevitably dampens down public concern for how to cut down on the smaller scale accidents.

A frequent rallying cry amongst those concerned with hazards is that as technology grows so does the risk. Even the CSS will say in its working party's report 'popular concern about risks has increased recently, as man-made disasters carry the threat of even worse to come from new or projected technologies'. A more technological world may indeed open up the possibility of human error causing more deaths; the six hundred deaths on Tenerife would have been only one hundred from a similar accident twenty-five years ago. But all in all the fatality rate for air travel has been steadily declining during this same period that the potential for bigger tragedies has been growing, so maybe the intro-

duction of new technology, some of which is safety-oriented, has had a beneficial effect.

Because things have improved in one well-studied industry doesn't, of course, mean the same will happen everywhere, and it is obviously right that we should go on asking more and more searching questions about safety. But modern scientific analysis coupled with intensive computer simulation of hazards can easily give the public a false impression that by better and deeper study the present-day technologist can give more and more assured guarantees that anything newly introduced will be 'safe'. As accidents from the trivial to the severe show, however, human actions, logical in themselves but illogical in a broader context, are at the heart of many disasters. These actions can never be properly simulated—who would have put a candle into the computer program for safety at Browns Ferry?

What we have to do if we really want the benefits of new technology is to be prepared for some mistakes to find their own way out—a principle, incidentally, followed throughout the whole process of scientific advance.

Obviously we don't allow a few stored nuclear weapons to go off by accident in order to learn how they can be safely stored, but in many environments, such as factories, air traffic, coal mining, novel engineering structures and so on, it is only with the help of an occasional accident, however distressing, that some very important and valuable pieces of information ever emerge. In an age in which the public expects scientists and technologists to think of everything ahead of time, this idea of learning from mistakes is often forgotten. But to learn effectively, the after-the-fact enquiry must be very wide ranging, because accidents, even road accidents, frequently result from a string of errors, sometimes apparently of little relevance. Thus one requirement, insufficiently widely discussed, in risk reduction is that the scope of enquiries should not be too severely circumscribed. This is a political matter and decisions are often taken against a background of fears of a 'fishing expedition'; maybe there is some good research to be done on how wide committees of enquiry should spread their nets. □

When is a drought a drought?

Michael H. Glantz and Richard W. Katz, of the Environmental and Societal Impacts Group at the National Center for Atmospheric Research, Boulder, Colorado, draw on the case of the Sahel to comment on the use of the term 'drought' for arid regions

THE word 'drought' has various interpretations: decision-makers, political and otherwise, underestimate its likelihood in and around regions where interannual rainfall variability is relatively high. In such arid and semiarid areas, recurrent droughts are in fact a part of climate and not apart from it, and therefore should not be viewed as unexpected events.

The West African Sahel, one such area, has recently been affected by its third major extended drought this century. Many observers have suggested that the drought, which began in various parts of the region in 1968, ended with a return to normal or near normal rainfall. Some interpreted the relatively good rains in 1974 as indicative of a resumption of 'normal' rainfall. Similar statements have been common in the growing volume of drought literature. However, they can mislead because they imply that a return to normal rainfall, that is, rainfall seen as an 'average', is associated with a return to acceptable if not favourable meteorological, agricultural and social conditions for the region. The validity of such an assumption is questionable.

What is perceived as 'normal' rainfall is an important, yet often overlooked, aspect of what is meant by 'drought' in arid and semiarid regions. People react to their perceptions whether or not those perceptions are based on reality, and the consequences of those reactions are real. It is misleading to view 'normal' as a 'normative quantity' with the implication that a departure is 'abnormal': a 'normal' has no predictive value. The literature concerned with droughts in arid and semiarid regions is replete with references to particularly dry years as being abnormal or anomalous. Yet, because perceptions about what con-

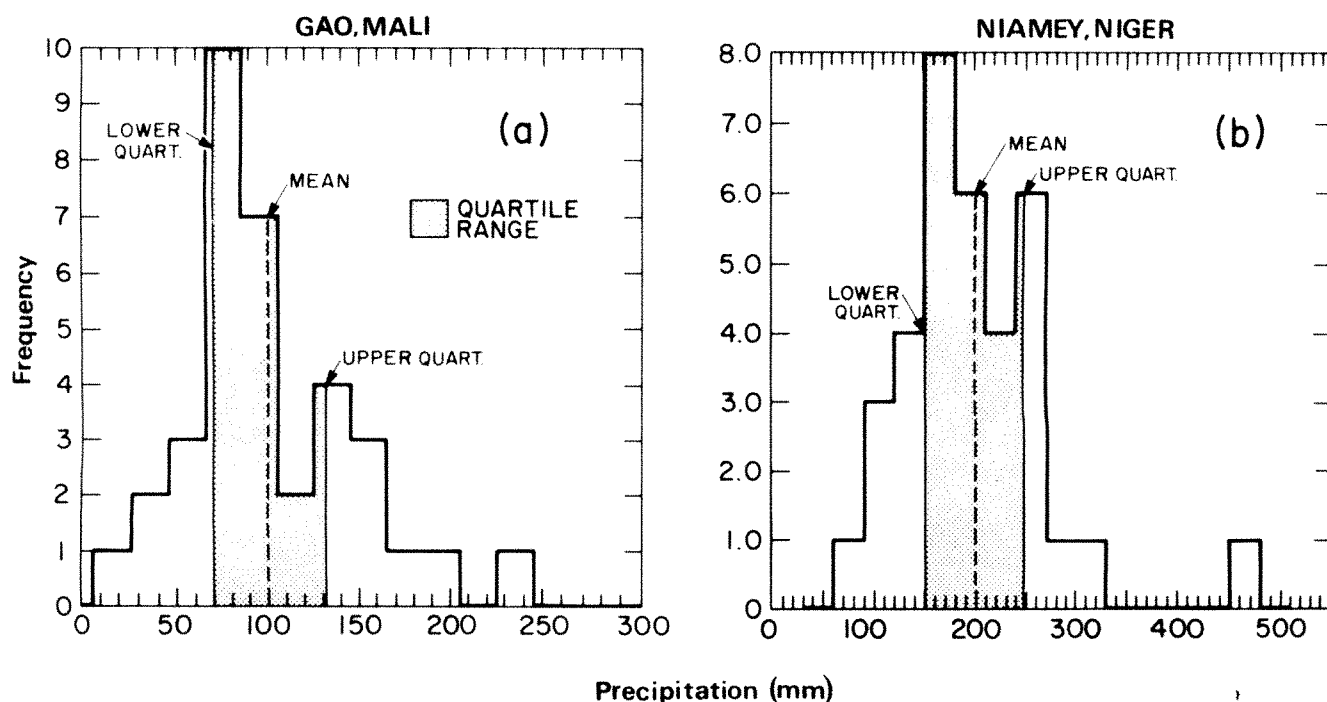
stitutes a drought vary, there is no widely accepted definition of drought. The scores of existing definitions, however, are either meteorological or agricultural. A meteorological drought could arbitrarily be defined as that period when the amount of precipitation is less than some designated percentage of the long-term mean, whereas an agricultural drought could be defined in terms of the timing of the rainfall which is crucial to crop development.

In reality, the subjective distinction between a dry spell and a genuine drought is a difficult one to make. Most American farmers do not call a dry spell a drought until the moisture shortage has seriously affected the established economy of the region. These observations apply equally well to similar situations in arid areas. It has been suggested that there should be at least two components to a definition of drought: a physical one and a social one. A mix of dry spell and meteorological drought occurred at various times and various places in the Sahel between 1968 and 1974, but their impacts were worsened by the pressure of populations, both human and livestock.

A major problem associated with the perception of drought is the constantly changing nature of those perceptions. Recent weather tends to influence perceptions more heavily than earlier weather and wet spells more heavily than dry ones. On this basis it has been suggested that the inhabitants of the Sahel have adopted practices during the abnormally wet years which end in disaster during the dry years. After a period of abnormally high rainfall in the Sahel which ended about 1965, the inhabitants considered a return to average conditions for rainfall to be a 'drought'.

What, then, does a return to average rainfall conditions in arid and semiarid regions mean? Of what importance is it when a return to 'normal' or near normal rainfall is perceived to be a return to drought-like conditions? Take the case of the Sahel, a climatic zone in sub-Saharan Africa which receives 200–600 mm of annual mean rainfall. It is bordered on the north by subdesert (100–200 mm) and to the south by the Sudan (600–900 mm). Rain falls during

August precipitation 1941–75



convective thunderstorms over a four-month period beginning in June. It is this highly variable rainfall on which the region's inhabitants depend for the cultivation of their crops and the maintenance of their herds.

August rainfall data are significant in that August is nearly always the wettest month and it marks the midpoint of the rainy season. We have looked at rainfall statistics for arid zones by taking the August rainfall records for two cities within the Sahel—Gao, Mali (16.3°N, 0.1°W) and Niamey, Niger (13.5°N, 2.1°E)—and have found that Sahelian rainfall shows a relatively high degree of variability. Many analyses, however, have not included a plot of the actual data but have relied on the use of descriptive statistical terms such as the mean or the running mean. Such terms fail to reveal magnitude and variability which are major factors in understanding the nature of rainfall in arid and semiarid lands.

Histograms derived from the data for Gao and Niamey show a 'positively skewed' frequency distribution, small amounts of rainfall occurring more frequently than relatively large amounts. This type of skewness is characteristic of the rainfall distribution not only in the Sahel but, more generally, in arid and semiarid regions. In particular, the degree of skewness is greater the drier the climate.

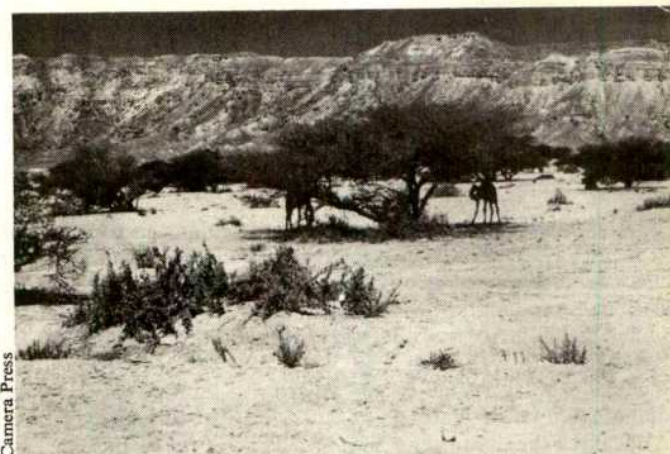
The mean, which is commonly taken to represent 'normal' rainfall, is extremely sensitive to skewness because it is inflated by the relatively few cases of large values: more than half of the observations fall below the mean. As a result, rainfall observations close to the mean value do not occur often in the Sahel, showing that, at least in some sense, the mean is too large and not at all indicative of how much rain commonly falls.

The median marks the amount of precipitation which is exceeded one half of the time, while the mode represents the amount of precipitation which is most likely to occur. Since neither of these measures is greatly affected by skewness, their interpretations remain clear. In particular, for a positively skewed distribution they are usually smaller in value than the mean.

For the Sahel, therefore, the mean, in reality, is above 'normal', while the median and the mode can still be interpreted, in the sense just discussed, as 'normal'. Hence although most analyses of Sahelian rainfall assume that the mean is an appropriate measure of central tendency, either the median or the mode may, depending on the circumstances, be preferable and no single number can adequately represent the distribution of Sahelian rainfall, given the large deviations from each of the measures of central tendency. 'Normal' rainfall, however, can be better characterised by the interquartile range than by any of the standard measures of central tendency.

Measuring the variability of Sahelian rainfall is further complicated by the possibility of trends or cycles or other evidence of climatic change in the data: there is strong disagreement among researchers over whether significant trends or cycles do exist. Although they might well exist, there may have been an inordinate degree of effort devoted to 'hidden periodicity' research and this has tended to obscure the most important aspect of Sahelian rainfall—its extreme variability. In fact, even if trends or cycles do exist, it is almost universally agreed that they would be of limited predictive value.

Aside from the absolute amounts of rainfall, as measured over varying lengths of time, other factors such as spatial distribution, timing, wind velocity and evapotranspiration could have an important impact on the social value to the inhabitants of the rain that does fall. If rainfall information, which represents only one climatic variable, is not viewed within its total environmental context, it can be extremely misleading. Where long-range development planning is concerned, such an oversight can be disastrous. Such has often been the case in fragile ecosystems like those in



Camera Press

arid and semiarid regions.

Desertification—the creation of desert-like conditions where none had existed before—is the result of either the vagaries of weather and climate or the mismanagement of the land or, as in most cases, some combination of both. Such ecological deterioration in the Sahel has been linked in several ways to the increased sizes of livestock herds. During extremely favourable periods of rainfall, as was the fifteen-year period preceding 1968, the pastoralists moved into the marginal regions in the north with relatively large herds. However, with the onset of a series of dry years beginning at the end of the rainy season in 1967, the pastoral populations found themselves overtaxing very marginal range-lands with the result that the nomads viewed themselves as victims of a natural disaster.

Deterioration and ultimately desertification in the Sahel and in similar ecosystems could be combated if an ecologically realistic carrying capacity for the rangelands was determined. Although there appears to be widespread acceptance that such a determination would be significant, there has been little agreement to date on how to make operational the notion of carrying capacity (defined as the amount of grazing stock that the pasture can support without deterioration of either the pasture or the stock). Should the carrying capacity be geared to the best, the average, or the poorest years? Which combination of statistical measures would be most meaningful for the planning of long-term development of rangelands? On which variables should such an assessment be based—vegetation, rainfall, soil, ground and surface water, or managerial capabilities? Such inconclusiveness within the scientific community, while understandable, creates confusion for the decision-makers, who often decide either to take no action or who decide that all scientific suggestions are of equal weight and, therefore, indiscriminately choose any one of those suggested. Given the downward spiral of land deterioration it becomes essential that an ecologically acceptable carrying capacity be established and enforced.

The mistaken idea that drought in the Sahel is an unexpected event has often been used to excuse the fact that long-range planning has failed to take rainfall variability into account. People blame the climate for agricultural failures in semiarid regions making it a scapegoat for faulty population and agricultural policies. It will therefore be crucial that decision-makers know what statistical and quasi-statistical measures actually mean: no single number can adequately describe the climate regime of an arid or semiarid region. Decision-makers must supplement such terms as the 'mean' with more informative statistical measures to characterise adequately the variability of the climate. An understanding of this high degree of variability will serve to remove one of the major obstacles to resolving the perennial problems of the Sahel and of other arid or semiarid regions. □

Going on the defensive

Constraints on the UK chemical industry are affecting research in its laboratories. **John Manos** describes how

THE chemical industry has suffered some nasty shocks in recent years. The basic economics of oil-based petrochemicals production has been severely shaken by rises in the oil price, while in other sectors the industry has had to face the onslaught of environmentalists and regulators.

Because the industry is more research-based than most, it is not surprising that these pressures should have had their effect in directing work in its laboratories. But they are not the only factors which have contributed to a major shift in research aims. In general, a growing maturity in the private industry's end products together with the above constraints (and the economic pressures of a recession) have meant that research effort is increasingly described as 'defensive', allowing companies to maintain their existing positions in the face of controls and inflation, while consolidating their expertise in established product areas.

Research is increasingly devoted to 'replacement' ventures, in which old processes, extravagant on energy and raw materials, are replaced by new processes economical in both. By applying a total design or total systems concept, the industry is re-training itself to satisfy this new long-range research aim. Also the paucity of totally new products which have provided a satisfactory return over the decade, has meant that less and less effort is devoted to 'blue sky' research and the research function is having to gear itself to satisfy more of private industry's short-term aims. That means fewer new drugs and pesticides and no really new plastics.

This applies to both those polymer products which showed spectacular growth over the past twenty years and to the higher value fine chemicals, drugs and agrochemicals whose development is more readily associated with broadly-based laboratory research aims. In both these areas less than 10% of research effort is now spent on the search for new products, the increasingly competitive nature of the business now requiring that 90% be spent on improvement and widening the range of products and on reducing costs.

The bulk petrochemicals industry, which has allowed the rapid growth of polymer production to take place, has reached a different sort of maturity. The petrochemicals industry believes

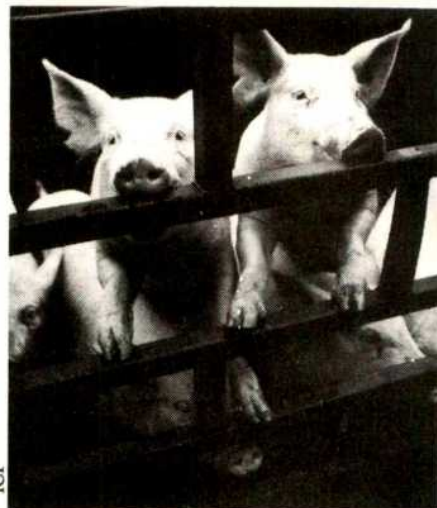
that Europe should have an energy policy that preserves for the chemical industry the chemical structures in 5% of total oil production, oil being too valuable to burn. If governments behave foolishly, the industry in Europe is considering which raw materials will replace (and to what extent) the cheap naphtha refinery by-product on which it has grown up. Research here is economic analysis of how best new feedstocks can fit in with established downstream production. While governments and state industries look at (or talk of) long term possibilities for the exploitation of new bulk raw materials, such as associated gas from North Sea oil wells or even coal, private companies are pre-occupied with justifying the operation of their existing facilities.

Biochemical engineering

If there is a pattern characterising research trends that is not symptomatic of the search for energy economy, pollution-free processes, or quickly exploitable product refinements, it is the opening up of entirely new areas of business based on the extension of chemistry into biology, and chemical engineering into biochemical engineering. It is surprising, for example, that each of the UK's big three chemical companies (and one of West Germany's) should have devoted such great research effort in seeking to move into the animal feedstuff industry. Yet this illustrates one of the chief areas of diversification: biochemical engineering.

In commercialising their bioproteins (or single cell protein) processes, BP, ICI and Germany's Hoechst are entering into a commodity business which is less well-known to them. In fact it is uncertainty about future price trends of the natural products which they aim partly to replace—soya bean and fish meal—which the third UK company, Shell, cites as the reason for its withdrawal from the development race. Nevertheless, 20% of ICI's 1977 capital investment sanctioning is devoted to a 50–75,000-ton-per-year plant to produce pig and poultry feed from hydrocarbon (methanol) feedstock. Methanol is also used in another animal feed development of ICI's at Billingham.

The bioproteins development programme has cost ICI £10 million over the past eight years with half spent in the last three. In its 1977 report, ICI



'Pruteen' piglets

describes this as a result of its 1960s initiative in applying process engineering know-how to microbiology. It is part of a bio-sciences research policy which the company has increasingly pursued in cooperation with universities. Other products of this line of research include processes for the continuous production of enzymes for the food industry (particularly isomerase for the production of high fructose corn syrup, for which ICI is now building a US manufacturing plant) and ICI's 'deep shaft' waste water treatment process which uses a mildly pressurised biochemical reaction to accelerate the conventional treatment process.

The forces which have encouraged the majors to venture into such unknown areas are the same as those which make research management in existing areas of business so difficult: the need for speedy exploitation of new developments countered by the increasingly long lead times to get new chemical products on the market. This latter point has been well documented for the agrochemicals industry where resources required to carry new insecticide through its seven- to nine-year path of screening, toxicology, field trials, registration and marketing are such as to restrict innovative development to only a small number of major companies.

Development effort

The development effort has swung over to what are generally less toxic products such as herbicides, or to the development of more efficient methods of applying existing compounds: ultra-fine sprays, slow release and encapsulated insecticides. Also newer synthetic pyrethroids under development are safer than the organophosphorous and carbamate products which are themselves safer than the organochlorines (which include DDT, aldrin and dieldrin).

Plant design and construction has been one area where companies have attempted to shorten development time. Shell Chemical, for example, has invested £19 million in a flexible agrochemicals production facility at Pernis, Rotterdam, which, although initially dedicated to specific products, incorporates in its design a service and equipment flexibility which will allow a switch to production of another product in as little as two months. But Shell still regards agrochemical development as a high-risk area from which resources will be diverted to more profitable business unless the regulatory pressures are eased.

The need to accelerate the worldwide exploitation of new developments is cited as one of the reasons why the overseas research budget of ICI has increased in recent years (from 25–30% of total in 1976). Thus, in the USA, ICI has extended its pharmaceuticals research effort so as to have the advantage of a scientific dialogue across the North Atlantic; and in India it is hoping to set up some central research facilities that will better use indigenous Indian raw materials within the Indian economy.

In general, however, rationalisation means centralisation, as illustrated by Hoechst and Shell's plans for their research facilities in Europe, although ICI still believes in a great deal of autonomy for its separate major business area. The greater part of Hoechst's investment in research facilities in recent years has been in its new multidisciplinary centre being built up in Frankfurt, which the company feels is necessary to cope with the rapidly changing demands of the market and of raw material supplies. Similarly last year Shell completed the transfer of all chemicals and polymers research facilities outside North America (except for agricultural pro-

ducts) to one site in Amsterdam.

As with agrochemicals, polymer research trends have been greatly influenced by the need to consolidate positions of strength and refine the properties of mature products. Hoechst notes that of the twelve plastics which represent 90% of Germany's consumption, not one has been developed within the last ten years. Polypropylene is the fastest growing new bulk plastic which threatens to compete strongly with the ethylene-based polyethylene, PVC and polystyrene (which account for 80% of German consumption) but this good rate of growth has come after twenty years of development! Other unique new materials such as carbon fibre, the wonder material of the sixties, have been overtaken by existing polymer technology which can satisfy rapidly changing customer demands more quickly and cheaply. The day of the accidentally-produced polymer having a market developed for it have passed.

The same accent on the selective development of established products was echoed by Shell last year in opening its polymer research centre in Amsterdam. It was suggested that the company, which had already withdrawn from two polymer areas (general-purpose rubber in the USA and high density polyethylene in the UK), might withdraw from other areas if its position was not strong. Certainly some polymer areas have shown a poor profitability recently. But as a number of products reach maturity over the next few years, Shell hopes that the concentration of product-oriented research capability at Amsterdam will allow a broadening of the range of applicability while process research is directed towards the development of improved, or second-generation processes for the production of the same polymers.

Non-productive research

Perhaps the most rapidly growing research area, however, is that involving non-productive work associated with toxicology and the control and detection of carcinogens. The complaints about over-regulation now heard from the agrochemical sector come more than a decade after the first problems associated with the use of agricultural chemicals caught the public eye. It was in the 1950s that concern over such products as the insecticide DNOC (dinitrocresol), which had caused the deaths of four farm workers, resulted in the setting up of the UK's Pesticide Safety Precaution Scheme. But increasingly stringent screening and animal testing techniques have always played a major part in product development in the agrochemical and the pharmaceutical industry. Exposure

limits and precautions for use of other chemicals not in contact with people or vegetation have generally been retrospective, based on experience of the chemicals in use.

This will not be so in the future. The 'ecology movement' and the need to avoid the difficult problem of dealing with a widely-used and manufactured product which is subsequently found to be dangerous and/or persistent in the environment will mean far tighter control on the introduction of all new chemical products. The US industry is already grappling with the massive requirements of the Toxic Substances Control Act and similar legislation is presently being formulated in Europe.

One of the biggest problems is the control of cancer-causing substances, or those which have given indication that they might be carcinogenic. The furore created by the US Food and Drug Administration's recent restrictions on the use of the sweetener saccharin and plastic soft drink bottles containing acrylonitrile indicates, according to the more pragmatic European view, the disadvantage of adopting a strict legalistic approach to the problem. But whatever the control strategy employed with known carcinogens, the problem remains simply how to screen new products for carcinogenicity to avoid the problem arising. With the several thousand new chemical products introduced every year being required to satisfy this sort of criterion, there is an urgent need for a simpler, quicker and cheaper screening procedure in preference to the conventional lengthy animal studies.

One of several possibly applicable *in vitro* tests is the Ames test, developed in the USA, which uses the mutation of bacteria strains as an indication of possible carcinogenic potential. For US companies such as Dow Chemical, currently spending 15% of its R&D budget on toxicology, such developments are of major importance in the light of new legislative requirements. But in Europe, too, *in vitro* testing is a major research area: ICI's central toxicology research laboratories have further developed the Ames test (in combination with another test involving mammalian cell transformation) to give the most reliable *in vitro* indication of carcinogenicity yet developed.

This exciting project is already justifying a budget of £250,000 a year which will rise to about £750,000 a year by 1978–9. Through such techniques, the company also sees a possible resolution of the problem of establishing no-effect levels for exposure to carcinogens, possibly within five years. □



Protein pilot plant

USA

Space in a corner

A tough fight is in prospect over two important space science projects for which funding is threatened. Colin Norman reports from Washington.

THE budget for the National Aeronautics and Space Administration (NASA) has run into unexpectedly heavy weather in its passage through Congress, and two major scientific projects could be in trouble. They are the Large Space Telescope, an orbiting optical telescope which has long been regarded by space scientists as NASA's top priority space science project, and the Jupiter Orbiter Probe, a proposal to launch twin spacecraft early in 1982 to orbit Jupiter and send probes into the planet's atmosphere.

A key subcommittee in the House of Representatives has already voted to block funds for the Jupiter project, and a sharp attack on the space telescope is expected in the Senate, with Senator William Proxmire leading the charge. Proxmire occupies a pivotal position since he is chairman of the subcommittee which is handling NASA's budget in the Senate; he is also armed with a new analysis suggesting that the total cost of building and operating the space telescope could run as high as \$1,400 million.

Though it is possible that both projects could be rescued by an intense lobbying campaign, already being mounted by NASA and its clients in the scientific community, loss of either of them would be a serious blow to the space science programme.

Both projects have been on the drawing board for some time, and NASA is now seeking Congressional approval to move them into the development stage. It has requested \$36 million for the space telescope and \$20.7 million for the Jupiter Orbiter Probe in fiscal year 1978. Those sums represent the first instalments toward the total development costs of \$435 million for the telescope and \$285 million for the Jupiter project, according to NASA's latest estimates.

Opposition from Senator Proxmire to the space telescope has long been anticipated, but the moves against the Jupiter project seem to have caught NASA officials by surprise. The first sign of trouble came late last month, when a House appropriations subcommittee deleted funds for the project from NASA's budget request. The subcommittee is said to have been unwilling to allow NASA to begin two

major projects in one year, and since subcommittee members had been under heavy pressure from space scientists to approve the telescope, they opted for that project and voted to kill off the Jupiter mission.

The subcommittee's recommendations will be reviewed by a meeting of the full House Appropriations Committee next week, but it is considered unlikely that the funds for the Jupiter project will be put back at that stage. Similarly, it would be unusual for the full House to overturn the committee's recommendation by restoring the funds. NASA is therefore concentrating its lobbying efforts on the Senate in the hope that if the Senate approves the project, House members can be persuaded to follow suit when differences between the House and Senate versions of the NASA budget bill are eventually settled in a House-Senate conference committee. The crucial vote will come in mid-June, when Proxmire's subcommittee is due to vote on NASA's budget bill, and the outcome is difficult to predict for a variety of reasons.

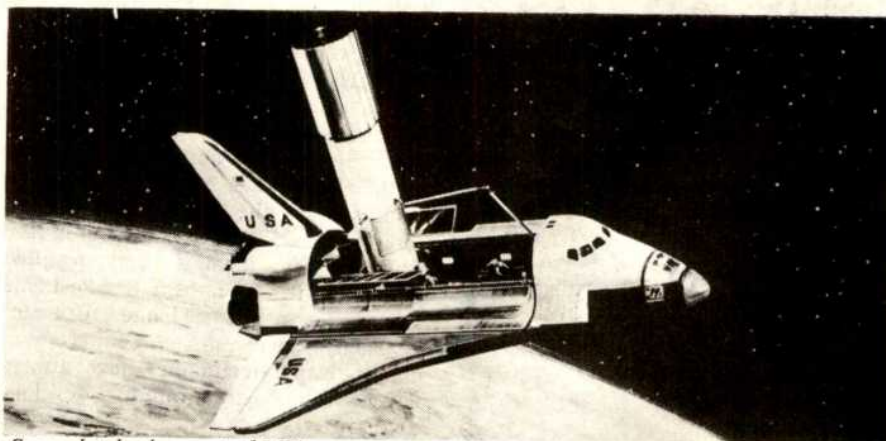
The chief focus of attack in the Senate is likely to be the space telescope, and Proxmire has already fired his opening shot. The Large Space Telescope (LST) has been under serious consideration by NASA since the early 1970s, and it has been consistently and enthusiastically endorsed by astronomers. Due to be launched by the space shuttle in 1983, the LST would have a major advantage over ground-based telescopes: the light it receives would not have to penetrate the semi-opaque curtain of the Earth's atmosphere, which filters out all but a narrow band of colours and blurs distant objects. As a result, the LST would be able to 'see' objects about 10 times smaller than those that can now be picked up

by the most powerful ground-based instruments, and it would be able to search for objects emitting light from the far infrared to the far ultraviolet, a range one thousand times larger than Earth-bound instruments.

Though the telescope has always been popular among scientists, it has not received unqualified support from Congress. A couple of years ago, before NASA had even asked Congressional approval for the LST, the House Committee on Science and Technology directed the agency to seek international participation in the project, to help defray some of the costs. Consequently, NASA has established a European connection. Last October, the Science Planning Council of the European Space Agency (ESA) approved a contribution of some \$80 million to the project, and according to NASA officials, a formal agreement will soon be signed under which ESA will supply a faint object camera, a solar array, a number of scientists for the project team, and assistance in maintaining and overhauling the telescope.

The LST has already been considerably delayed. NASA was originally hoping to begin building the instrument this fiscal year for launch in 1982, but the project collided with President Ford's attempts to hold down government spending. The Office of Management and Budget deleted funds for the LST from NASA's fiscal year 1977 budget before the budget was sent to Congress. According to NASA officials that delay has already hurt the project and a further delay would probably kill it. It would be difficult to keep the design teams together for much longer, they argue, and European support would probably melt away if Congressional approval isn't assured.

Proxmire opened his assault on the LST last week by releasing a report prepared by the General Accounting Office (GAO) which suggested that the



Space shuttle placement of LST

total cost of building the telescope and operating it for its projected 15-year lifetime could amount to \$1,400 million. "The country faces an \$80 billion deficit, and it is time the Congress said 'no'. The space telescope is a good place to start", said Proxmire.

The GAO essentially took NASA's estimate for \$435 million to develop the LST, and added \$96 million for inflation, \$65 million for tracking and data acquisition, \$470 million for general operations costs, \$81 million for civil service personnel, and \$211 million for shuttle launches to put the instrument into orbit and to refurbish and maintain it.

The GAO figures have sent NASA officials into orbit. In a letter printed in the GAO report, Noel Hinners, Associate NASA Administrator for Space Science, charged that "presenting this eclectic array of cost data as a life-cycle cost estimate for the space telescope is misleading". In an interview with *Nature* last week, Hinners said that NASA is still estimating that the development cost for the LST will be about \$435 million, and that the operations costs will be about \$10-\$15 million a year. The operating estimates do not include shuttle launch and refurbishment, he acknowledged, because it is not known how frequently the telescope will be visited for maintenance.

Hinners argued that the operating cost for the LST will not be much different from the \$10 million it costs to operate a major ground-based facility such as Kitt Peak. "I don't see anybody saying that if Kitt Peak lasts for 100 years it will cost \$1 billion", Hinners wryly observed.

Nevertheless, whatever the accuracy of the GAO figures, the report highlights the fact that the cost of the LST will not end with the launch, and the figure of \$1,400 million is sure to be a powerful weapon in Proxmire's hands. Few people are willing to predict the outcome in the Senate for, although Proxmire has failed to sway his committee against NASA projects in the past, there has been a significant change of membership on his committee this year. Two staunch supporters of the space programme, Lawton Chiles of Florida and Bennett Johnston of Louisiana, have been replaced by James Sasser of Tennessee and Patrick Leahy of Vermont, both of whom are fiscal conservatives. Even if he loses in committee, however, Proxmire is expected to take his fight to the Senate floor.

According to committee sources Proxmire is concentrating on the LST at this stage, and is probably prepared to approve funds for the Jupiter Orbiter Probe (JOP). But the sources

suggest that if Proxmire loses on the LST, he may then turn on the Jupiter project. NASA officials would regard loss of that project as a body blow for the planetary science programme in general and to the Jet Propulsion Laboratory in particular. If both the House and Senate delete funds for the project, there would be no chance to reinstate them in conference committees.

At present, there is only one approved planetary mission under way. That is the so-called Voyager project which involves launching spacecraft in August this year to flit past Jupiter and travel on to Saturn. One of the spacecraft may also be re-targeted to swing past Uranus eight years after launch. Thus, if the JOP is not approved, there would be a major hiatus in the planetary programme, with no projects under development at all. According to NASA officials, up to 300 people would be laid off at the JPL, which is now the focus of NASA's planetary work. It should be noted that if the 1982 launch date is missed, there would not be another good opportunity until 1987, because Jupiter will be too far away.

Again, NASA officials argue that if the JOP is killed, planetary science teams would be broken up, and the continuity between programmes would be lost. The technology used on the Pioneer Venus probes would be adapted to the JOP mission, and the JOP spacecraft technology would in turn be used on the planned out-of-ecliptic mission. If there is a major disruption in the planetary programme, "we would lose that inheritance from one mission to the next", Hinners says.

NASA officials are taking those arguments to Senators now in the hope of building support for the efforts. Though the prospects are difficult to assess, it is expected that the LST will scrape through in the Senate, and that the Senate will also approve some funds for the JOP. If those decisions are upheld in the conference committee, NASA would be able to start work on JOP next year, and avoid major layoffs.

Finally, it should be noted that the reason why these projects are deemed especially crucial this year is because some space science projects have been squeezed out of NASA's budget during the past four years to accommodate funding for the space shuttle. Space scientists have been assuming, however, that when funding for the shuttle begins to run down, more money would be made available for space science. Next year is the first year of reduced shuttle funding, but science projects are still at the front of the firing line. □

DNA bills drafted

UNDER intense lobbying from a number of scientists, committee staff members in the House and Senate have put together draft versions of legislation to control recombinant DNA research in the United States. Though there are still plenty of opportunities for the drafts to be altered by the committees themselves, major differences are shaping up in the approaches embodied in the House and Senate versions. The draft Senate bill, in particular, is causing consternation among many scientists.

The House bill has already been approved in principle by the health subcommittee of the House Commerce committee, and members of the subcommittee are expected to meet next week to approve the final wording. It will then go to the full committee for approval before being sent to the floor of the House. The Senate version has not progressed that far. It has been drafted by staff members of the health subcommittee, though it is said to reflect the views of the subcommittee chairman Edward Kennedy, and it will not be considered by the committee itself until next week at the earliest.

The House draft would require the Secretary of Health, Education and Welfare (HEW) to draw up new recombinant DNA regulations, with the advice of a top-level advisory committee, and to licence facilities where such experiments are to be conducted. The bill would leave much of the implication of the regulations, including the granting of most licences, to local biohazards committees, however. Consisting mostly of scientists, the committees would include lay members and representatives of local communities. The Senate version, on the other hand, would establish an eleven-member commission in HEW to draft regulations and issue licences for facilities and for individual experiments. Both versions imply that, until new regulations are adopted, guidelines issued last year by the National Institutes of Health would be given the force of law.

The Senate approach has come under heavy fire by scientists, most conspicuously in a resolution approved late last month by the National Academy of Sciences, for fear that it would lead to bureaucratic delays and subject the research to inordinate red tape.

One of the most controversial issues in the debate on federal legislation has been whether state and local governments should be allowed to establish local controls which are more strict than the federal regulations. The draft House bill would allow them to do so only if they can prove to the Secretary of HEW that there are compelling local reasons for stricter controls, or that the federal regulations are not strict enough to protect public health, requirements which come close to making the federal controls paramount. In the Senate, Kennedy is said to favour allowing state governments to set stricter controls, though some other members of his subcommittee favour the House approach and agreement has yet to be reached.

Colin Norman

BRITAIN

Waiting for summer

Chris Sherwell reports on latest developments in Britain's nuclear power debate, now picking up again

THE issue continues to insinuate itself; only its locus changes. The excitement over nuclear power in recent weeks—since President Carter announced his policy—has been generated in various international forums, notably in London at the meeting of the so-called nuclear suppliers group and at the Downing Street economic summit, and in Salzburg, where two conferences were held in quick succession.

Now the issue is coming home. The second Salzburg conference had not even ended late last week before British experts were called away to attend one of the two meetings which took place in London at the weekend on domestic nuclear policy. The one bringing the experts back was called by Mr Anthony Wedgwood Benn, the UK Secretary of State for Energy, and was held in secret. Ironically, the other meeting, held in public and involving hundreds of largely lay people, attracted far less publicity. It was organised by Friends of the Earth (FoE).

Called a conference for a non-nuclear world, the FoE meeting produced a mostly young audience whose views on nuclear power were much as expected: negative. Their enthusiasm was fanned by remarks from two MPs, Robin Cook and Nigel Forman. Cook urged local groups to latch on to nuclear-related local issues and to exploit them relentlessly; Forman's advice was simple—citing a recent opinion poll, he said "secure yourself the help of a friendly scientist".

Neither man was invited to Benn's meeting, though the Commons was represented, through the chairman of the Select Committee on Science and Technology, Arthur Palmer. Indeed, many of the 30 or more people meeting in the seclusion of the Civil Service College at Sunningdale were favourably disposed to nuclear power. They included Sir John Hill of the UKAEA, Con Allday of BNFL, Ned Franklin of the National Nuclear Corporation (NNC), and Frank Tombs of the Electricity Council. Others present included Sir George Porter, Sir Alan Cottrell, Lord Todd, Sir Herman Bondi, Sir Kenneth Berrill, Lord Balogh, Dr Walter Marshall and Sir Brian Flowers—altogether a high-powered line-up.

That not all were so disposed—Walter Patterson of FoE was there to

put the alternative view—at least said something about Mr Benn's desire to hear all sides of opinion. And opinions were what he was there to hear: it was not a meeting to take decisions; rather, it was a meeting that anticipated them.

After months if not years of the public debate he has himself spurred on, Mr Benn is now coming under increasing pressure to call a halt to it all and to start, in the words of one participant at Sunningdale, doing what he was elected to do—take decisions. With the inquiry on an oxide fuel reprocessing plant at Windscale for BNFL due to begin on 14 June, that controversial issue is temporarily out of his hands. But two others await action.

One is on the choice of nuclear reactor for Britain, an issue which goes back to at least 1974, when everyone thought it was finally agreed that the Steam Generating Heavy Water Reactor (SGHWR) would be the country's next generation reactor. The issue was reopened last year, and decision-time has again arrived. The choice is between the SGHWR, the Advanced Gas-Cooled Reactor (AGR), a British product now apparently performing well after teething troubles, and the Pressurised Water Reactors (PWR) which now dominate the world market.

Press reports over the past couple of months have revealed a tilt in favour of the AGR. Mr Benn is himself awaiting a detailed report from the National Nuclear Corporation on the

three types of reactor, and has advised its compilers of the sort of questions he wants answered. He is also awaiting a report from the National Nuclear Inspectorate on the safety of PWRs. Both reports are due out soon, probably next month.

At the weekend meeting Sir Brian Flowers, the former chairman of the Royal Commission whose report last year on nuclear power became the layman's *locus classicus*, urged that a choice be made. That report, the government's response to which is also due to be published shortly, escalated the debate on the fast breeder, which is the subject of Mr Benn's other decision. Having originally promised a decision last autumn and then stated earlier this year that a decision yet is not necessary, Mr Benn is now talking about the coming autumn as a deadline. The decision is on whether to go ahead with a demonstration commercial fast breeder (CFR1) at Dounreay, and the industry, notably in the form of Sir John Hill, is saying that there is no time to waste.

All the suggestions of urgency, and there are now many, do not derive from an immediate need for energy. The worry is that the growing surplus Britain is now acquiring will be lost by the 1990s, and that long lead times make action now essential if nuclear power is to play its allotted role alongside coal and conservation as one of the principal originators of Britain's supplies in the 1990s. That nuclear power is destined to do that was apparently agreed at Sunningdale; that it should entail a full nuclear cycle and

After Moscow, the world

EVERYONE knows the world market for energy has been transformed in this decade. Not everyone appreciates that the world market for energy (and every other commodity) could be transformed completely and even more radically within the next couple of decades if China and the USSR, separately or together, produce the sort of economic growth that countries like Japan or Germany have experienced.

One man who does claim to appreciate the problem is Britain's Secretary of State for Energy, Mr Anthony Wedgwood Benn. Last week he spent two full days in Moscow and came away enthralled. His purpose was to paint in the detail of the 'eastern perspective' on the global energy canvas that he likes to speak about. To get the other perspectives—west, north and south—he has already visited Washington, Norway and Saudi Arabia. Having assimilated all that, his next step will come at the EEC Council of Energy Ministers in the middle of June, when he reports back.

His tentative conclusion is that there is a need for a world energy policy.

What this means precisely is not clear: to many, a world policy implies a world government to implement it; to Mr Benn it means at the very least having information on world supply and demand for energy. He cites almost scornfully a book out this week on global energy prospects to the end of the century which takes no account of countries like China or the Soviet Union.

So what did he learn in Moscow? That the USSR is the world's biggest energy producer. That the Russians hope to use still higher voltage electricity transmission. That they are interested in Britain's offshore oil technology. That they do not dump medium-level nuclear waste into the sea. And that they are very keen to hear assessments of US nuclear policy.

Mr Benn also received one "small rebuke". Each year, he was told, 100 US scientists visited the USSR, and 100 from the USSR visited the States. For France the figure was 70. For Britain over a three-year period, however, the figure was only 50.

Chris Sherwell

a system of fast breeders was a matter of greater dispute.

From all accounts the participants agreed that if reprocessing was to be done at all, it could be done most reliably at Windscale. There was also fairly wide agreement that technically speaking enough could be done to combat the threat of proliferation, but that the problem of public acceptability—the all-important political dimension

—would remain. Had any of the participants retained doubts on that score, the other weekend meeting might have helped to dispel them.

Now, with few new arguments to present or to hear, everyone with any influence over the shape of Britain's nuclear policy is hoping for just one thing: that with decisions ultimately a matter only of a signature, Mr Benn will have a restful summer. □

SWITZERLAND

Guidelines emerge

Rosmarie Waldner reports from Zurich on Swiss moves to produce guidelines for genetic manipulation experiments

A SWISS Academy of Medical Sciences Committee founded in 1975, the Commission for Experimental Genetics, recently took the first step towards regulating genetic manipulation in Switzerland when it sent a circular to all researchers in the field of recombinant DNA recommending observance of the guidelines laid down by the US National Institutes of Health (NIH), Switzerland. It sent a circular to the guidelines issued for NIH researchers in June last year. The Swiss guidelines, however, are not legally binding, although they are considered to be obligatory for the researcher. The committee also wants research projects to be registered, in line with the recommendations of the Strasbourg-based European Science Foundation (ESF).

The commission, under the presidency of Professor Werner Arber of the Biozentrum of the University of Basle, is of the opinion that "things as they stand at present do not justify issuing an official order. The caution expected of researchers in this field, with its legal basis in the Epidemic Law, should ensure general observance of the recommended guidelines". The Swiss Epidemic Law regulates measures in connection with infectious diseases and is one of the few laws in the field of public health valid in all parts of Switzerland; public health, not being a federal matter, is under the jurisdiction of Switzerland's 25 cantons.

At their annual conference held in early April the Swiss Society for Cellular and Molecular Biology discussed the guidelines and voted overwhelmingly in favour in them. However, some speakers demanded that the guidelines be made legally binding and that the public should be brought into the discussion. Altogether six laboratories at the universities of Basle, Geneva and Zurich and at the im-

munology institute of the pharmaceutical company Hoffman-La Roche in Basle are working with recombinant DNA.

The commission is available for advice or will arrange for expert assistance to be provided by the European Molecular Biology Organisation (EMBO) in Heidelberg if local researchers have any doubts about the correct classification of a research project within the various criteria of the NIH guidelines. It is also available for general consultation and for the interpretation of the NIH guidelines. As Professor Arber stresses, the commission does not consider the NIH guidelines adopted in Switzerland as unalterable; it wants to keep a constant watch and make changes (even if these deviated from the NIH guidelines) if this was considered necessary.

When registering, researchers responsible for a project must announce their intentions and state the precautionary measures taken. "The committee considers the observance of these measures, registration and the provision of correct information to colleagues to be part of the duty of each and every researcher", the circular states. Research heads and heads of department at universities and other public and private institutions have been requested to support the commission's efforts.

The Swiss National Fund for the Promotion of Scientific Research particularly wants to promote the research in Switzerland. When it comes to the allocation of research funds, however, it insists on observance of the guidelines. The fund has mentioned the possibility of becoming involved in the establishment of a special safety (P4) laboratory. This project is expected to cost SF1.5 million.

The Swiss press has welcomed the guidelines, but there have been complaints that decisions concerning research with such far-reaching consequences should have been made in camera—the commission has no lay representatives, nor even experts from

W. GERMANY

Breeder hold-up

UNDER increasing pressure from environmental protectionists, the West German government has called a temporary halt to research and development on the fast breeder reactor. Last week, Dr Hans Matthöfer, the West German Minister for Science and Technology, announced that no more money was to be spent on R&D for the fast breeder until questions raised by the Social Democrats on the safety of fast breeder technology and the proliferation of nuclear weapons through the spread of plutonium had been considered by the Bundestag.

This means a bar on spending whatever has not yet been committed of the DM122 million set aside for fast breeder R&D under the government's medium term energy plan. Work already contracted, however, such as the prototype liquid metal fast breeder at Kalkar, will go ahead. It will be financed from that portion of the DM227 million fast breeder programme not devoted to research. Other projects unlikely to be axed are West Germany's collaboration in the construction of the French Superphénix and the fabrication plant for fast-breeder fuel elements which is already in operation.

Prior to the latest announcement discussion about the further development of breeder reactors in West Germany was becoming livelier. In the Social Democratic party above all there was increasing resistance to further financing of R&D work. The Ministry for Research and Technology had explained in detail to the Bundestag the pros and cons of the evolution of the fast breeder—it seems unsuccessfully.

Germany began development of the breeder in 1960—researchers got their early experience through participation in the construction of the American experimental reactor SEFOR. The 300 MW breeder at Kalkar is a German-Belgian-Dutch joint project due to be completed in 1982. Within the framework of the German-French agreement on Superphénix, Germany contributes 16% of the cost via electricity supply undertakings.

When the West German government presented its energy research programme at the end of April, Dr Matthöfer claimed that because of public resistance, his ministry had felt obliged not to balance the programme as much in favour of nuclear power as it had originally intended. It had been necessary to cut the target of 45,000 MW of nuclear power by 1985 to 30,000 MW. Nevertheless, the energy research programme puts greatest emphasis on nuclear power: out of the DM6,200 million to be spent on energy up to 1980, DM4,500 million was to go on nuclear energy. □

fields other than biology and medicine. The hope was expressed that parliamentarians would soon be roused: questions were put in parliament as early as two years ago; since then there has been silence on the subject.

IN BRIEF

US nuclear snag

President Carter's plans to defer nuclear fuel reprocessing and to turn down the breeder reactor programme have hit a snag in the US House of Representatives and they could face a stiff challenge in the Senate. Last week, the House Committee on Science and Technology voted 38 to 0 to keep funds for a demonstration breeder reactor in the budget for the Energy Research and Development Administration (ERDA), but with the understanding that the committee will investigate the matter further and vote on the funds again before sending the ERDA budget bill to the House floor.

Committee sources note that the vote was a procedural measure which doesn't necessarily indicate the committee's final view, though they suggest that the prevailing opinion among committee members seems at present to favour rejecting Carter's request to cancel the demonstration project. In the Senate, Senator Frank Church, who heads a key energy subcommittee, said in a speech at MIT earlier this month that Carter's plutonium strategy will not work because other countries are unlikely to follow suit. In a later interview with the *Washington Post*, Church said that the United States should continue with the breeder project and with reprocessing to avoid

"nuclear isolationism". The opposition to Carter's plans virtually guarantees that there will be a major debate on plutonium policy on the floor of the House and the Senate this summer.

Pipeline report

Controversial recommendations from a Canadian judge regarding proposed pipelines stretching between the Arctic and Southern Canada and the USA will not be acted upon at least until a second report is published, probably in the summer. The judge, Thomas Berger, reported on the ecological and social implications of the proposal last week, and recommended a morator-

THE heavy veils of secrecy that the French are drawing over the details of their method of enriching uranium to reactor- but not to weapons-grade make it difficult to evaluate their claims. But if their recently revealed chemical enrichment technique stands up to full scale testing and is as tamper-proof as they suggest, cheap enrichment plant should be available in the 1990s to many countries which might not have been considered responsible enough to own it.

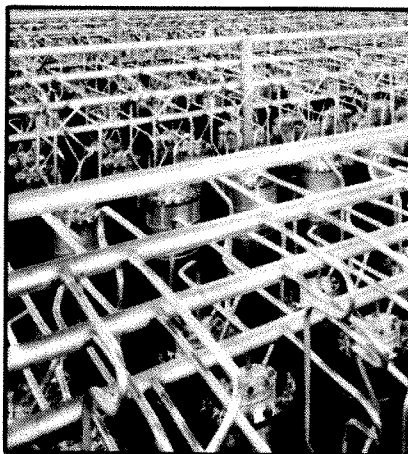
The French announcement, at the recent IAEA nuclear fuel cycle conference in Salzburg, was neatly timed to coincide with the economic summit in London. With an undoubted shortage of enrichment plants the new process seems to offer the possibility of meeting the need without increasing the risk of proliferation. It is not clear, however, whether such enrichment facilities would allow the use of the fast breeder reactor to be postponed; that depends on whether, in the absence of the new technique, the use of uranium in thermal reactors would be limited by a shortage of enrichment facilities or by the scarcity of uranium itself—which in turn will be determined by the economics of extraction.

Enrichment used to receive a great deal of attention as a process encouraging nuclear proliferation. Concern has since focused on reprocessing the spent fuel. Natural uranium, containing 99.3% ^{238}U and 0.7% fissile ^{235}U , must be enriched to 3–4% ^{235}U for use in American LWRs and 2% for the British AGRs. The Canadian CANDU and the British Magnox reactors use natural uranium, as oxide and metal respectively. The sensitive aspect of enrichment is that an enrichment plant may be arranged so that enrichment to over 90% instead of 2–4% is

achieved, enough to make a uranium bomb.

Reprocessing offers a similar opportunity, but here to make a plutonium bomb. The civil purpose of reprocessing spent reactor fuel—that is, separating plutonium from uranium and radioactive wastes—is to use the

Power sans bombs



Enrichment cascade (Fotosmit)

BACKGROUNDER

plutonium to fuel more reactors and especially the fast breeder. The French development could make it more difficult to get hold of uranium bombs, but would not affect the ease of access to plutonium bombs.

The main enrichment process in current use is gaseous diffusion, uranium hexafluoride being diffused through a porous membrane. The lighter ^{235}U atoms diffuse slightly more readily than the ^{238}U and enrichment is achieved by a large number of stages in cascade. Compression and cooling at each stage mean that energy consumption is very high (6% of the energy generated by PWR fuel). In the newer ultracentrifugation process, favoured by the British, Germans and Dutch, the

diffuser is replaced by a centrifuge. The capital costs of centrifugation are very high but the electricity consumption is only 10% that of diffusion.

The chemical exchange process announced by the French is said to have the advantages of low energy consumption, rather simple technology and suitability for small-scale plants. Separation of the two uranium isotopes based on the different rates with which molecules containing them react has been explored in the past, but had been largely discounted by most workers because the very small difference in chemical reaction rate only allows extremely slow separation.

The French breakthrough apparently lies in finding two uranium compounds which undergo reactions whose rates are more markedly affected by the uranium isotope. What the molecules are has not been revealed. The enrichment process is still rather slow but this is the main safeguard against bomb manufacture; while the period of two years needed to produce 3% enriched uranium by the chemical method seems industrially feasible, it would take fully 30 years to produce bomb-grade plutonium. By contrast, bomb-grade material can be prepared in two years by gaseous diffusion and one day by ultracentrifugation.

The French say two other safeguards are built into their process. One is that the process cannot be arranged in cascades (which can, at least in theory, be manipulated to produce a small quantity of 90% enriched fuel instead of a larger quantity of 3% enriched fuel). Furthermore, if too much enrichment is attempted, the uranium risks going critical. This seems to be because a liquid phase is involved, and in a liquid there is the risk of local concentrations of very high enrichment.

ium, to the chagrin of industrial interests. A second report on the economic need for the pipeline is awaited from the Canadian National Energy Board, which regulates the oil and gas industries and is still holding hearings.

USSR action on seminar

Professor Mark Azbel has been ordered by the Soviet authorities to cease holding the 'Sunday Seminars' for refusenik scientists. The seminars have been held regularly for five years, and only a month ago Professor Azbel was given a once-off permission to hold a special anniversary session—the first time that the authorities openly acknowledged the existence of the seminars.

Within the past three weeks, Pro-

fessor Azbel has been summoned three times to the Lubyanka and Lefortovo prisons for interrogation. He was obliged to sign an undertaking not to reveal the contents of the investigation. It is known, however, that it related to his association with Anatolii Shcharanskii, the unofficial 'press-agent' of the seminar group at present held for investigation for alleged contact with the CIA.

Last week Dr Viktor Brailovskii and Dr Veniamin Fain, who are among the senior members of the group, were also called in for interrogation.

The most recent message received from Professor Azbel was that the authorities are striking at the 'Professorate'. Previous action against refusenik scientists has been aimed at the lower echelons.

NPL's new director

Dr Paul Dean is to take over as director of the National Physical Laboratory (NPL) on 1 October. He will succeed Sir Iuean Maddock, who has been acting director since giving up his appointment as chief scientist at the Department of Industry at the end of last March. Dr John Dunworth, the previous permanent director, retired last June; he joined the NPL as deputy director in 1962 and became responsible for the laboratory in 1964. Dean joined the laboratory in 1957, and will be moving from the Department of Industry's headquarters, where he was head of the Space and Air Research division. He was also Head of the Research and Development Contractors division.

THEY came in busloads from as far away as Minnesota. They wore pro-laetrile buttons. They were prepared to pack the hearing room, to applaud wildly at the inflammatory oratory of their leaders, and to boo, hiss and interrupt when members of the 'medical establishment' warned them of the uselessness of laetrile in cancer therapy.

The occasion was the Food and Drug Administration (FDA) Administrative Rulemaking Hearing on Laetrile, held in Kansas City, Missouri at the beginning of May. To 'bend over backwards', the FDA had talks by the laetrile crusaders, following opening remarks by the presiding officer. My experience with hearings led me to know that the television cameras and reporters would be present at the morning session, and they would depart at noon, leaving the opponents of laetrile without media coverage, just as the opponents of banning DDT were treated in 1969.

Efforts at appeasement always fail. At 8 am on the first day the laetrile pushers called a press conference that was largely devoted to rhetoric against the FDA and allegations that the hearings were a "kangaroo court". I asked if the laetrile supporters had been given the opportunity to speak at the hearings. The answer was, necessarily, "yes", but a woman hastened to my side and hissed in my ear that if I interrupted their press conference with any more questions, she would call the hotel manager and have me removed forcibly from the room. I managed to avoid this fate when I asked my next question, by alleging that I was a reporter for *Nature*. I requested any scientific literature reference, even one, to substantiate the claim that laetrile is

vitamin B17. I was told "wait until tomorrow".

During the hearings, professors of medicine were jeered at and heckled. Advocates of laetrile, shouting and declaiming in the manner of a

Quackery triumph



THOMAS H. JUKES

religious revival meeting, were wildly applauded as they testified to their "cures". The audience was ecstatic, as well it might be. Bills to legalise laetrile without even defining what laetrile actually is, have just been passed by the legislatures of Alaska, Florida, Indiana and Nevada. The Indiana Bill had successfully overridden the Governor's veto. The Nevada bill includes a 10% cut on laetrile sale for the State Treasury.

The publication of the laetrile pushers, *The Choice*, was bursting with exultant information. It contained advertisements for "Reverend Clifford Oden's stirring call to faith in God and vitamin B17", for a new

water distiller that would remove fluoride from drinking water, and for a book on "chelation therapy in the killer diseases". The publication informed its readers that "efforts to decriminalise laetrile use in Texas surged into the forefront where 150 members of the lower house shouted their approval of the freedom of choice bill and stood up and cheered after the unanimous vote was delivered". One of the photographs showed two 'Wisconsin committee leaders' carrying "23,000 names on signatures" in support of pro-laetrile bills introduced into the Wisconsin legislature; another was of Gloria Swanson telling her health philosophy and her personal victory over a tumour.

The pro-laetrile campaign is administering a crushing defeat to science and rationality. Undoubtedly it is well-financed. A Californian 'laetrile physician' was indicted on charges including one of depositing \$2,500,000 in California banks, May 1973 to August 1975. His licence was revoked for "gross negligence and incompetence", 29 November, 1976.

The technique for arousing support is an appeal to revolt against authority, bureaucracy and the 'medical establishment'. Political action is effected by writing letters to legislators. This procedure was overwhelmingly successful with the Proxmire Bill in the US Senate 1975, specifically directed against the authority of the FDA.

Things fall apart; the centre cannot hold; Mere anarchy is loosed upon the world, The blood-dimmed tide is loosed, and everywhere

The ceremony of innocence is doomed; The best lack all conviction, while the worst

Are full of passionate intensity.

W. B. Yeats

correspondence

Technology and politics

SIR,—After making several criticisms of *The Technology of Political Control*, your editorial (14 April, page 577) ends with a conclusion which displays an apparent ignorance of the structural basis of control. Although I accept the admirable sentiment that, "the real need is for scientists who are involved in military and police research to be able to speak up when they see the balance shifting in an unsavoury direction," the realities of the situation in practice make this suggestion naive. Given the essence and ramifications of such work, it is likely to be covered under the Official Secrets Act and thus government researchers in these areas are forbidden from making their work public.

The writers of the *New Technology of Repression* are among the few scientists who can present unrestricted information on this topic in an informed way. Yet, as the recent search of my house and university room by the Special Branch indicate; no-one who researches in this controversial area can be assured of freedom from interference. It must not be forgotten that scientists are also numbered amongst the victims of the technologies of political control.

Yours faithfully,
STEVE WRIGHT

Department of Politics,
University of Lancaster, UK

'The Selfish Gene'

SIR,—It is no great surprise that W. D. Hamilton has written such an intemperate letter about my review of *The Selfish Gene* (12 May, page 102). Despite his considerable concrete contribution to the theory of kin selection, Dr Hamilton has himself given way to the lure of unscientific speculation on the biological basis of human history, and is responsible for his fair share of vulgar Darwinising. I will comment on three points in his letter that have appeared frequently in replies to criticisms such as mine. First, despite a string of pejorative comments on my objectivity and the quality of my analysis not a single instance is offered of my mistaken analysis. The reader will be as suspicious as I am that "It would be easy to defend the book and reply to the review point by point" when not a word of defence is offered, but

only 350 words of angry invective.

Second, with great modesty, Dr Hamilton compares himself and his fellow sociobiologists to Darwin, and Dr Dawkins to Huxley. Other biological determinists have recently, in their apologies, compared themselves to Galileo! Everyone with a pet theory thinks himself a Darwin or Galileo, the moment his errors are criticised. But my criticism of Dawkins comes not from an outraged church establishment, but from the solid accumulation of the science of population genetics. The real problem in scientific progress is to distinguish those with a revolutionary new insight into nature from crackpots with delusions of grandeur. Self-advertisement is poor evidence.

Finally, Dr Hamilton confesses that he feels little concern about the basic epistemological questions that are at issue. Rather he feels a great warmth about the "spirit" of Dr Dawkins' book and the "science" it describes. But it is precisely the substitution of "spirit" for the elements of logical thought that makes *The Selfish Gene* such a worthless piece of vulgarisation. What is revealed by Dr Hamilton's letter is that Dawkins' errors are not simply those of an overenthusiastic but naive populariser. They are an accurate reflection, it seems, of the quality of thought of the sociobiologists themselves. We will surely not understand the nature of the world by an excess of spirit and a deficiency of hard thought. Dr Hamilton, Bishop Wilberforce would have been proud of you.

Yours faithfully,
R. C. LEWONTIN
Museum of Comparative Zoology,
Harvard University,
USA

Scotland get its share

SIR,—It is difficult to know whether the letter by D. N. M. Hamilton (28 April, page 770) is meant as a serious contribution or as a joke. If he seriously thinks that those who advise the Medical Research Council state that the Scots believe that the people in London are more deserving of money for research than are the research workers in Scotland, I should have thought he would have drawn this to the attention of the MRC directly and suggested most strongly that they change their advisers.

My department has been generously

supported and assisted by the MRC on the basis of projects submitted to them, and in my many conversations with the administrators in the MRC, I have been left in no doubt that there is considerable regard for the immense amount of scientific activity that is conducted in the scientific centres in Scotland. At no time during any conversation have I been left with the impression that institutions outside London—and particularly those North of the border—are regarded as poor relations. If there should at any time appear to be a departure from the strict correlation between the percentage of population and percentage of MRC money spent in a given area, I suggest that the remedy lies with Dr Hamilton and others who may think with him, to produce research and other programmes which are judged to be well worth supporting from the limited funds which are available to the various grant-giving bodies.

I am not an adviser to the MRC, nor has my view on the distribution of their funds ever been sought, and, in common with the vast majority of research workers, I also have had programmes rejected, but one keeps trying.

Yours faithfully,
P. J. HEALD

Department of Biochemistry,
University of Strathclyde, UK

A plea for hyphens

SIR,—At the risk of sounding pedantic I want to make a plea for a return to the sound practice of writing a hyphen in compound words used adjectivally. Today's general practice of omitting the hyphen in such adjectives as 'hormone-induced' and 'temperature-sensitive' is sheer sloppiness and, more seriously, often leads to ambiguity. That almost everyone does it is no excuse. Gowers, to whom lip-service is still being paid, gives very sound advice on the matter of hyphens on page 183-84 of *The Complete Plain Words* (HMSO, London 1973). So do several English and American style manuals and dictionaries.

Yours faithfully,
J. FABER

Hubrecht Laboratory,
Utrecht,
Netherlands

news and views

The structure of transcribing chromatin

from H. R. Matthews

THE bulk of eukaryotic chromatin is organised in a repeating subunit structure. Each subunit or nucleosome contains a core of two each of the histones H2A, H2B, H3 and H4 with about 195 base pairs of DNA folded or coiled outside the histones into about $\frac{1}{2}$ of its extended, B form, length. The nucleosome can be reconstituted from DNA and histones alone and in this structure the DNA is not available for transcription, at least not by *E. coli* RNA polymerase. Tissue specific non-histone proteins can modify the DNA and histone structure to give active chromatin where the DNA is accessible to RNA polymerase (see for example Paul & Gilmour *J. molec. Biol.* **34**, 305; 1968). The same situation probably applies *in vivo* and so although the bulk of the DNA in chromatin is coiled in nucleosomes what is the structure of active DNA?

Isolation of active chromatin is an important step towards studying its structure although this road is littered with scientific retractions. The other two approaches are to look for active genes in nucleosomes and to study chromatin in the electron microscope. J. M. Gottesfeld and his colleagues have described a promising approach to the preparation of an active chromatin component which involves digestion of total chromatin with spleen DNase II followed by precipitation of inactive chromatin with $MgCl_2$. The Mg^{2+} -soluble chromatin has been found to contain nascent RNA, to include DNA sequences that are known to be transcribed, but to exclude inactive DNA sequences. This has been shown in Friend cells which can be stimulated to synthesise globin messenger RNA. Mg^{2+} -soluble chromatin from unstimulated Friend cells does not contain globin genes whereas Mg^{2+} -soluble chromatin from the stimulated cells does. Moreover, in a range of cell types the amount of Mg^{2+} -soluble chromatin correlates with the overall transcriptional activity. The Mg^{2+} -soluble chromatin displays the same DNA

repeat length, 198 base pairs, as bulk chromatin which implies that the DNA repeat is a property of a class of chromatin structures rather than of a unique structure. However, complete nucleosomes from the Mg^{2+} -soluble chromatin sediment faster than nucleosomes from the bulk chromatin. Mg^{2+} -soluble nucleosomes contain RNA and non-histone proteins as well as histones and are particularly sensitive to pancreatic DNase I. Preliminary experiments suggest that Mg^{2+} -soluble nucleosomes contain regions of unstacked DNA, unlike the bulk nucleosomes (Gottesfeld, Royal Society Discussion Meeting, 1977). Under the action of RNase the Mg^{2+} -soluble nucleosomes change into the bulk nucleosome structure so Gottesfeld's results are interpreted to mean that active chromatin is in a different structure from bulk chromatin but that the active structure readily changes to the bulk structure.

In the second approach bulk nucleosomes are prepared and their DNA is hybridised to cellular mRNAs to try to detect active genes. Considerable hybridisation is observed suggesting that active DNA is indeed in the bulk nucleosome structure, but these experiments are also open to the alternative explanation that in the cell population only some copies of the genes tested are active at a given time and the hybridisation could be due to nucleosomes released from inactive sequences. Reeves (*Science* **194**, 529; 1976) suggests this may be at least partly the case from his data on the proportion of rRNA genes found in bulk nucleosomes from cells with different rates of rRNA synthesis. As the table shows, rRNA genes are under-represented in nucleosomes from cells where rRNA synthesis is high.

The rRNA genes are relatively easy

to study because they are normally highly active and reiterated but in the past few years the availability of pure eukaryote mRNAs and reverse transcriptase (RNA-dependent DNA polymerase) has allowed studies of genes such as those for globin and ovalbumin which occur in only one or a few copies per genome. These genes are also found in bulk DNA from nucleosomes so they also exist in the nucleosome structure or in a closely related structure.

Bulk nucleosomes are prepared with micrococcal nuclease which produces double-strand breaks in DNA. Another nuclease, DNase I, produces single strand breaks at 10 base-pair intervals in DNA in chromatin to give products that are of single strand lengths 20, 30, 40 . . . 160 or more bases when analysed under denaturing conditions. Weintraub and Groudine (*Science* **193**, 848; 1976) and G  rel and Axel (*Proc. natn. Acad. Sci. U.S.A.* **73**, 3966; 1976) have studied the digestion of the globin and ovalbumin genes respectively. The globin gene is highly sensitive to digestion by DNase I in adult erythrocytes but in fibroblasts where it is inactive the globin gene is less sensitive, as is the bulk of the DNA. The differential sensitivity was confirmed in different cells for the ovalbumin gene. DNA complementary to mRNA was used as a probe for the presence of ovalbumin genes in DNase I digests of liver nuclei where the ovalbumin gene is not expressed, and in DNase I digests of oviduct nuclei where about 75% of the ovalbumin genes are active. These important experiments show that active genes are in a different structure from the bulk of the DNA and from inactive genes although other data suggest the 'active' structure must be closely related to the bulk nucleosome structure.

<i>Xenopus laevis</i> cells	Complement of rRNA genes	Rate of rRNA synthesis per gene	% rRNA genes in bulk nucleosome DNA
Adult erythrocytes	+ / +	Minimal	86
Log phase embryonic tissue culture cells	+ / +	Normal	72
Stage 46 larvae	0 / +	Maximal	60

Electron microscopists were involved at the very beginning of the discovery of nucleosomes. What can they tell us about the structure of active chromatin? Chromatin can be seen after spreading by the Miller technique and the nucleosome structure survives although some stretching of the DNA between nucleosome cores can occur. In chromatin from nucleoli that are actively synthesising rRNA the nascent RNA molecules are packed very closely together in association with spheres of 10 nm diameter along the DNA. Are these spheres RNA polymerase complexes or nucleosomes? W. W. Francke's group (Scheer *et al.* *Chromosoma* **60**, 147; 1977) have measured the length of the transcription unit in pictures of active rDNA and compared it with the length of the largest RNA product that can be isolated. If this is the primary transcript then the comparison shows that active rDNA must be extended in the B form and not packed into nucleosomes. A further correlation has been noted between the total rDNA repeat as measured along active chromatin and

as determined by restriction nuclease analysis, which implies that the non-transcribed spacer is also not in nucleosomes. These are very striking results but some other electron microscopists, notably McKnight and Miller (ICN-UCLA Symposium on Molecular Human Cytogenetics, 1977) have electron microscope data which they interpret as showing the presence of nucleosomes on active chromatin. More work is needed to resolve the apparent disagreement. The electron microscope appearance of active chromatin may depend on how closely packed the RNA polymerase complexes are along the DNA or, if the active structure is easily converted to the bulk nucleosome structure and *vice versa*, then the living state may be a dynamic equilibrium which can be 'frozen' in different ways by different techniques.

Study of the structure of active chromatin is clearly at a very early stage but it is already fascinating to piece together the data from different approaches and we can look forward to more exciting progress very soon.

example the review articles in the *Sunday NY Times*, sect. 4, page 6, 13 March; 1977). An earlier report of a panel set up by the US National Academy of Sciences, *More Water for Arid Lands* (NAS, Washington, DC; 1974), gives an excellent survey of water practices, both very new and very old, which enable the agricultural potential of arid lands to be realised.

Ancient solutions

Ancient desert dwellers collected rain by redirecting water running down hill slopes into fields or cisterns. Such agriculture was developed almost 4,000 years ago, and in the Middle East it permitted crop production on lands receiving as little as 100 mm average annual rainfall, thus sustaining agricultural civilisations in desert regions that today support only a small human population and produce few crops. In the Negev, traces of the Nabatean inhabitants remain in the rock walls they built along contours of hills to convey rainwater to lower lying fields, and in the gravel they cleared (piling it in mounds or strips) in order to bare the loess-like soils that form a crust when wet, increasing run-off. Modern versions of this latter technique use silicones, latexes, asphalt and wax to help make soils water-repellent and promote run-off. Contemporary estimates are that rainwater harvesting is possible in areas with as little as 50–80 mm average annual rainfall, although a water-harvesting catchment in Israel yielded a usable run-off in a year with only 24 mm of rain.

A variant form of rainwater harvesting is to build terraces or low walls on floodplains in order to catch and spread the flash floods that result from short, intense storms. These and other techniques have been developed by biologists and archaeologists at the Wadi Mashash, a run-off agriculture training centre in the Negev that serves trainees from arid lands all over the world. A more full account is given by Evenari *et al.* in *The Negev: The Challenge of a Desert* (Harvard University Press, 1971).

About 3,000 years ago the Persians dug qanats to bring mountain groundwater to arid plains. A qanat, an ancient Semitic word that is ancestor to "canal", is a gently sloping horizontal tunnel that brings underground water from the higher elevations of an alluvial fan down to the lower parts of the surface; a series of vertical shafts between the ground surface and the tunnel characterise the construction of a qanat, and can be used as wells and for maintenance. The old Persians built qanats on a scale rivalling the great Roman aqueducts. Many are still used, especially in Afghanistan and in Iran,

More water for arid lands

from Robert M. May

If the Earth were stationary, with the Sun circling round it, the global patterns of wind and weather would be simpler. There would be two convection cells, one in each hemisphere, with warm air rising at the equator, moving toward the poles, and then completing the convection pattern by returning at low elevations back toward the equator. The Earth's rotation introduces many complications, and broadly speaking the upshot is three convection cells in each hemisphere: in the first, air rises around the equator and falls back around latitude 30° N and S; the second cell, at intermediate latitudes, joins on, with poleward surface convection; the third cell has cold air falling at the pole and surface movement toward the equator. The interface between the second and third cells is relatively unstable, which contributes to the vagaries of the weather at high latitudes. These broad patterns in air circulation, and related patterns in ocean circulation, have had much influence on human history. (For example, they explain why it took so long for sailing ships to find the east coast of Australia, which was obviously a fateful event.)

As the air rises at the equator, it

expands and cools. In the process, water vapour is "squeezed out" (hence the cloud cover at the equator), and the air convected back to the surface at around 30° N and S latitudes tends to be dry. Mainly as a consequence of this fact, all the world's major deserts lie around $\pm 30^\circ$: in the south, the Kalahari, the Atacama (in Chile), western and central Australia; in the north the Sahara, the Arabian, Gobi, and Punjab deserts, and the Great Basin in the US southwest. This feature shows up in biogeographical patterns. Thus in De Candolle's classification of plant types, the smooth continuum from tropical (megathermic plants) to temperate (mesothermic), boreal (microthermic), and arctic (hekistothermic) zones is interrupted around $\pm 30^\circ$ by the band of xerophilic, or "dry-loving" plants.

As human populations grow, these arid regions pose increasingly difficult problems. Although the ultimate cause is not agreed, many of the world's sand deserts seem to be enlarging, and droughts are contributing to the economic devastation of whole nations. The six drought-stricken Sahelian nations probably provide the best known and most extreme illustration. Against this backdrop, the UN Water Conference met at Mar del Plata, Argentina, in early March (see for

where some 40,000 qanats comprise more than 270,000 km of underground channels supplying 35% of the country's water.

Modern alternatives

Beneath many of the world's deserts are reserves of saline water, which offer possibilities for the irrigation of suitably resistant crops. If leaching and drainage are adequate, water with total dissolved salts in the range 500–1,500 milligrams per litre can be used on all but the most salt-sensitive plants. Water with up to 3,000–5,000 mg l⁻¹ can be used with highly tolerant crops. The most salt-tolerant crop studied so far is Suwanee Bermuda grass, *Cynodon dactylon*, which can tolerate about 12,000 mg l⁻¹. This is still a long way from seawater, which has a total salt content of about 35,000 mg l⁻¹.

Reservoirs and canals in arid lands are subject to heavy evaporation losses. As a proportion of total water stored, such losses involve a surface-to-volume effect, and tend to be relatively most severe for large, shallow reservoirs and ponds. Increasingly ingenious methods are directed at covering the water surface with a barrier that inhibits evaporation: these embrace liquid chemicals, usually based on long slender molecules; waxes that melt in the sun to form a film; or solid blocks of poly-

styrene or other light material. Large reservoirs, lakes, and rivers are still beyond the reach of modern technology because it is very difficult for the evaporation-suppressing system to survive heavy winds, storms and floods.

Evaporation from the soil surface of irrigated crop systems can be similarly reduced by latex, asphalt, oil, gravel, or shredded plastic mulches. Alternatively, modern "trickle irrigation" methods apply small amounts of water at frequent intervals, to a specific site near each plant. Such techniques have proved most effective for permanent tree and vine crops, and for crops grown totally under cover. Trickle irrigation will benefit from technological advances that lower costs and increase reliability and freedom from clogging. Loss of moisture by percolation in sandy soils is a problem that can be ameliorated by producing artificial underground barriers of asphalt or plastic; again the difficulty is the cost.

In a typical plant, only 1% of the water absorbed by the roots is incorporated into the plant cells; the remaining 99% is transpired back into the atmosphere. In selecting and breeding crops suitable for arid lands, clearly one of the major goals is to minimise transpiration. But the choice of appropriate plants is another story (see for example *Nature* 265, 209; 1977). □

Vaccination against virus-induced tumours

from Robin Weiss

VACCINATION against Marek's disease (MD), a lymphoma of chickens, is one of the major success stories in viral oncology. It has aroused interest in the possibility of human vaccination against Epstein-Barr virus (a more dubious matter, I think). Marek's disease is caused by a highly contagious herpesvirus present in the feather-dust (dander) of chickens, and the acute form of the disease used to cause serious commercial loss to the broiler industry. Immunisation of one-day-old chickens with attenuated MD virus or with a related, non-pathogenic virus of turkeys protects the chickens from the disease (*Purchase Cancer Res.* 36, 696; 1976). Precisely how the vaccine strains confer resistance to MD is not understood, and two classes of antigens, virus-specific and tumour-specific, have been recognised. The Marek's tumour-specific antigens (MATSA) are

present on the surface of lymphoma cells, but not on MD virus-infected chick kidney cells which express viral structural antigens.

Inoculation of either killed tumour cells (Powell *Nature* 257, 684; 1975) or live, non-transformed, MD-infected kidney cells (Lesnick & Ross *Int. J. Cancer* 16, 153; 1975) immunises chicks against MD. At the recent symposium on 'Viruses and Cancer' held by the British Association for Cancer Research at Cambridge, Powell reported that he could dissociate anti-tumour immunity and anti-viral immunity to MD by different vaccination procedures. Immunisation of chickens with glutaraldehyde-fixed MD-infected kidney cells elicited humoral antibodies that neutralise the virus, whereas immunisation with fixed lymphoma cells elicited a cell-mediated anti-tumour cell cytotoxicity that does not affect viral replication. Both types of immunisation exerted a protective effect against the development of Marek's lymphomas.

Immunity to tumours caused by

RNA oncoviruses may also be accomplished by either anti-viral or anti-tumour cell activities, and there is much interest in preparing vaccines against feline leukaemia virus (FeLV) and its associated diseases. As with the Marek's disease MATSA antigen, FeLV-induced lymphomas express a cell surface antigen, FOCMA, which appears to be unrelated to the viral envelope glycoprotein, gp70 (Stephenson *et al. Proc. natn. Acad. Sci. U.S.A.* 74, 1219; 1977). Most of the effort in developing FeLV vaccines involves the use of FOCMA as the immunogen.

Recently however, de Noronha *et al. (Nature* 267, 54; 1977) report that cats can be protected from sarcomas induced by feline sarcoma virus (FeSV) when passively immunised with antibodies directed against viral envelope antigens. FeSV is closely related to FeLV and has the advantage for experimental studies of producing sarcomas that are lethal within 4 to 8 weeks of virus inoculation, according to dose. The antibodies were prepared in goats and reacted with either FeLV or murine Friend virus gp70. They neutralise FeLV and FeSV, and are cytotoxic to FeLV-producing cells. By inoculating cats with the goat antibodies after FeSV infection, the development of sarcomas was prevented or those that had already appeared regressed. Schäfer and his colleagues (*Bibliotheca Haematol.* 43, 86; 1975) have previously shown similar passive protection against Friend disease in mice treated with anti-gp70 serum. They propose that antibody-therapy could be widely applied to virus-induced malignancies.

One point usually ignored by investigators of oncovirus immunisation is that the tumours induced by the 'strongly-transforming' viruses grow mainly by infecting and transforming new cells rather than by proliferation of tumour cells. The importance of tumour growth by cell recruitment was clearly demonstrated by Pontén (*Natn. Cancer Inst. Monogr.* 17, 131; 1974) for Rous sarcoma in chickens, and it is also important for the development of Friend spleen foci and of sarcomas induced by FeSV and murine sarcoma virus (MSV). Indeed, spreading infection is necessary even in cultures of non-established embryonic fibroblasts for the appearance of MSV-transformed foci (Hartley & Rowe *Proc. natn. Acad. Sci. U.S.A.* 55, 780; 1966). While the prevention of tumour growth by antiserum to gp70 might be mediated through a cytotoxic effect on the tumour cells, an alternative mechanism may be through neutralisation of virus particles.

Linna *et al. (in Tumor virus infec-*

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tions and immunity (eds Crowell R. L., Friedman R., & Prier J. E.), University Park Press, Baltimore, 1976)) have shown by using a complex induction system of Rous sarcomas which demands virus rescue from virogenic, non-producing cells, that neo-hatching thymectomy increases the number of sarcomas, whereas bursectomy enhances their rate of growth without affecting tumour frequency. The effects of thymectomy and bursectomy on low-dose sarcoma virus inoculation might prove more illuminating for distinguishing between anti-virus and anti-tumour cell immunity.

With the weakly-transforming oncoviruses, such as the avian lymphoid leukaemia viruses or the murine AKR-Gross virus, tumour growth seems to be largely a consequence of proliferation of one or a few clones of leukaemic cells rather than progressive recruitment. In this situation, once the malignant clone has arisen, virus neutralisation should have no role in tumour control. However, anti-rival antibodies may be important after infection yet before malignant trans-

formation, as they clearly are in natural infection of chickens and cats. Even in mice, which carry the genomes of leukaemogenic viruses in all their cells as endogenous genetic elements, only those strains that produce re-infecting virus, such as AKR and C58, have a high incidence of lymphoma. Thus a humoral response against the virus may be efficacious in preventing lymphoma. Many strains of mice naturally develop humoral antibodies, and a recent paper by Lee, Ihle, and Huebner (*Proc. natn. Acad. Sci. U.S.A.* **74**, 343; 1977) shows that formalin-inactivated virus is suitable for eliciting antibodies to gp70 and p15E, the virus envelope proteins. It will be interesting to see whether such virus-immunised mice are protected against tumours and whether cell cytotoxicity or virus inactivation plays the more important part.

I suspect that both types of immunity will be significant. Their effects may well be synergistic, both in Marek's disease, and in oncovirus-induced leukaemias and sarcomas in chickens, cats and mice. □

smoothly shifts the peak absorption wavelength. The theme of this lecture was in effect the advent of optical absorption engineering, just as Wilson had described the engineering of energy gaps.

A. D. Yoffe (University of Cambridge) spoke about solids with one- or two-dimensional order, with special emphasis on electrical conduction. The tetracyanoplatinates featured again as variants of the chain conductor, but pride of place went to the polymer (SN)_x made by polymerising S₂N₂. This is the first known polymeric superconductor, with $T_c = 0.3$ K; however, at least one pair of long-chain eyebrows was raised in scepticism as to the polymeric status of this material. Attempts are being made to substitute Se for S and As or P for N with a view to improving the superconducting performance. Yoffe went on to discuss MX₂ layer compounds, a large family with M=Ti, Zr, Hf, V, Nb, Ta, Mo or Nb and X=S, Se or Te. These range from insulators through semiconductors to metals and show several kinds of order. Yoffe spent some time on Overhauser's notion of charge density waves which are well exemplified by some of these compounds. The CDW are linked with periodic displacements of atoms in the layers and in turn effect conductivity changes at the critical temperatures. Intercalation of metal atoms, such as K, between the layers can transform a metallic compound into a semiconductor or *vice versa*, and some intercalates turn out to be hard superconductors. There are prospects of developing some of these intercalates as superionic conductors for advanced batteries, and other applications (to magnetometers and as catalysts) were discussed. Much of this work stems from Yoffe's own group: research at the Cavendish on electrical conductivity of solids now spans the whole spectrum from highly ordered crystals to glasses.

A. R. Ubbelohde (Imperial College, London) demonstrated his enthusiasm for highly controlled pyrolytic carbon, especially the highly oriented, stress-annealed variety: collectively he denoted these materials as "pedigree pyrocarbons". He speculated about the band structure of this class of materials and, by extension, their potential as catalysts.

J. B. Goodenough (newly arrived in Oxford from MIT) spoke with great persuasiveness about the challenges facing the materials expert in the forthcoming burst of research on solar energy. He took his audience on a whirlwind tour of optically designed coated glass cover plates and microgrids for solar heaters, zeolites which show promise as adaptable water absorbers/desorbers for solar air-conditioning

Solid chemical physics

from Robert W. Cahn

A meeting on the Chemical Aspects of Materials Research was held in London on 21–22 March, 1977 under the sponsorship of the Chemistry Committee of the Science Research Council.

THE explicit aim of the meeting was "to stimulate greater activity amongst members of the present chemical community in certain areas of solid state science"—a praiseworthy purpose since in the British chemical family solid-state chemistry has been a poor relation, which some chemists are half-minded to show the door as an imposter. The word "materials" in the title was no doubt intended to stress the fact that this ill-defined branch of chemistry generates its fair share of industrial utility. The title of this report is as accurate a representation as any of what the symposium was actually about; "solid-state chemistry" would have been appropriate had there been more emphasis on reactions. Fuzziness at the borders of disciplines is a symptom of healthy activity: the SRC inevitably sometimes runs into demarcation problems, but makes amends for the occasional administra-

tive *longueur* by its sponsorship of genuinely interdisciplinary meetings such as the one under review.

The meeting consisted of ten hour-long lectures (a few of which are reported below) interspersed by generous discussion time, nowadays a highly untypical format. The value of the symposium was enhanced by the fact that speakers were able to follow their subjects in depth and also follow side-tracks.

B. L. Wilson (Plessey) opened the proceedings with a survey of the 'engineering' of highly alloyed semiconductors with adjustable bandgaps: (Ga, In, Al) (As, P). P. Day (Oxford) concentrated on unusual optical and magnetic phenomena, in particular ionic crystals in which optical absorption can be sharply altered by an applied magnetic field. FeCl₂ is the prototype: separate, ferromagnetically-bonded layers are coupled antiferromagnetically, and the anomalies arise when the layers become magnetically saturated and the salt effectively breaks down into two microphases, nucleation being catalysed by crystal dislocations. Other salts (RbCrCl₄, for example) have, at certain wavelengths, selective peak absorption, the magnitude of which is sharply dependent on magnetisation. In a third category, the tetracyanoplatinates, ionic substitution

(potentially a major application), and the conceptually difficult spectrum of phenomena ranging from straight electrolysis, through electrically assisted photolysis, to straight photolysis of water at electrically connected semiconducting electrodes without benefit of external batteries. (This whole field was initiated by a Japanese paper in *Nature* in 1972.) TiO_2 and SrTiO_3 have been tried, but other semiconductors with smaller bandgaps need to be developed, perhaps with dye sensitisers incorporated. The potential importance of this technology is very great.

In a beautifully lucid lecture G. Allen (Imperial College), basing himself on the Flory-Huggins insights, explained just why useful two-phase polymers can in general only be made by the stratagem of block copolymerisation, which alone permits the correct balance between the tendency to phase separate and the physical impossibility of doing so completely. To a metallurgist used to the interpretation of metallurgical phase diagrams in terms of the configurational entropy of crystalline solid solutions, the rationalisation of polymeric phase diagrams in terms of the configurational entropy of polymer chains was convincing. The laws of mixtures governing the physical properties of such systems were exemplified and the prospects for ungrafted solution-blended two-phase systems outlined. (Physically mixed polymer systems apparently have a Houdini-like proclivity for escaping from their most elaborate entanglements.) The formation of networks from T-shaped monomers was explained. This approach permits the design of tough structural plastics and rubbers, of materials with controlled water permeability and other special-function polymers. In this lecture the rationale of a lifetime's research strategy was made manifest.

The symposium was an unqualified success, and it is to be hoped that the SRC will feel encouraged to continue to sponsor such occasions. □

Green light for the signal hypothesis?

from Pamela Hamlyn

THE work on secreted proteins, particularly those of the liver and pancreas, has demonstrated that these proteins are synthesised on ribosomes bound to endoplasmic reticulum, then transferred through the membrane into the lumen so that their synthesis and secretion form a continuous process. What

is much less certain is the way in which the messenger RNAs which code for secretory proteins are selected for translation by membrane-bound ribosomes. That the selection may be a consequence of a particular nucleotide sequence in these mRNAs, or due to the activity of a separate class of ribosomes, are considerations which have found some experimental support, but the suggestion for which there is most experimental evidence is that it is a characteristic of the protein itself (namely the amino acids found at the amino terminus of a precursor form of the mature protein) which leads to its synthesis by membrane-bound ribosomes. A model for the mechanism by which the ribosome is brought in contact with the membrane by way of the N-terminal amino acids has been suggested by G. Blobel and his colleagues (for example, Blobel & Dobberstein *J. cell Biol.* **67**, 835; 1975).

Some very convincing evidence that it is the protein product, rather than the mRNA or ribosomes, which is responsible for the specificity of attachment of polysomes to membranes, has been produced by Harvey Lodish's group at the Massachusetts Institute of Technology (Wirth *et al. Cell* **10**, 253; 1977). Working with Sindbis virus-infected chicken embryo fibroblasts, they have isolated a 26S viral mRNA which codes for three proteins; one is a core protein which is eventually associated with the 42S RNA viral genome and the other two are glycoproteins required for the lipid bilayer which envelops the mature virus. The proteins are translated sequentially from one initiation site on the mRNA: first the core protein, then the two envelope proteins. This recent report is concerned with the localisation of the polysomes involved in the synthesis of these two classes of protein. It was found that 70% of the 26S RNA is bound to membrane and that newly synthesised core protein is localised on the cytoplasmic side of the endoplasmic reticulum and remains free in the cytoplasm, whereas the envelope proteins remain associated with membrane. Since the same ribosomes translate the same mRNA this is very persuasive evidence that neither the ribosome nor the mRNA determines which proteins are synthesised on membrane. In the model described by Wirth *et al.* it is proposed that at the beginning of translation the 26S RNA is free in the cytoplasm whereas the core protein is synthesised and released. As translation of the envelope protein proceeds, its amino-terminal sequence initiates an interaction with the membrane leading to the ribosome becoming 'membrane-bound'. No amino acid data is available

for the synthesised proteins but it has been observed that the first of the two glycoproteins is produced in a larger form than is found in the lipid envelope.

The light chain of mouse immunoglobulin has been known for some time to be synthesised in a larger form than is eventually secreted. This has been shown to be due to about 20 extra amino acids preceding the amino terminus of the mature protein. Israel Schechter and his coworkers have been working on the sequence of the amino acids in what they call the 'extra piece' in several different light chains (Burstein & Schechter *Proc. natn. Acad. Sci. U.S.A.* **74**, 716; 1977). Two completely sequenced, and other partially sequenced, 'extra pieces' reveal that that although these regions are not particularly homologous in sequence they all contain a very high proportion of hydrophobic amino acids. This fits nicely with the suggestion that this region is responsible for the interaction with endoplasmic reticulum leading to the ribosomes becoming bound to membrane. However, Burstein and Schechter envisage that the hydrophobic region would also allow the protein to associate with other membranes and suggest in particular that it may interact with the cell surface membrane to serve as an antigen-recognising receptor. This hypothesis can be tested experimentally: it will be interesting to see if the 'extra piece' is involved in several different protein-membrane interactions. □

Iranian natural history

from Peter D. Moore

THE past 30 years have seen an enormous expansion in amateur interest in natural history in Europe and North America. This surge has been accompanied and encouraged by a parallel proliferation of field guides for the identification of such organisms as birds, plants and butterflies. Taxonomy apart, these developments have been of great benefit to the serious study of distribution patterns, behaviour and migration. In many other parts of the world, where documentation is at present far less thorough, the galvanisation of the potential mass of amateurs may simply be awaiting the provision of accessible field guides to identification.

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In Iran, the government Department of the Environment regards such provision as an urgent priority. It began its programme of publishing field guides in 1975 with the production of *The Birds of Iran* (eds Scott, D. A., Hamadani, H. M., & Mirhosseini, A. A.). Many difficulties were faced in the production of that book. The choice of language (the book is written in Persian) the names used for birds in the country (selection from many local names had to be made) and the art work (illustrations from British and French field guides had to be used due to a lack of suitable indigenous artists) all presented problems. The book which resulted is impressive in its scope and the distribution maps are useful even to the non-Persian speaking visitor, if he is prepared to learn and use arabic numerals.

This year has seen the publication by the Iranian Department of the Environment of *A Guide to the Mammals of Iran* (ed. Harrington, F. A.). This time the text is in English and the art work, which has been splendidly executed, was specifically commissioned and was undertaken by R. S. Robinson. All 148 mammals recorded from Iran, including the tiger and the lion which are now probably extinct there, are described and 112 are illustrated. Iran covers a large range of geographical zones in its span between the Gulf and the Caspian and the arrangement of the book is particularly helpful in grouping species into habitat sections rather than using a strictly taxonomic order. In this respect the *Guide* innovates in a manner which may well be emulated in future western publications in this vein. Harrington's broad experience of the Iranian fauna is evident in the text and his work will undoubtedly pave the way for further studies, particularly on detailed geographical distribution in the country. Work is now underway on the next volume in the series which will deal with reptiles.

The difficulties and the needs of the botanist in Iran are rather different. The high central plateau is rich in endemics and the flora is therefore extremely large. A definitive *Flora Iranica* is still in the course of production, being edited and compiled by K. A. Rochinger at Vienna. At present, therefore, the production of popular field guides must await the full description of the Iranian flora, and new species are constantly being described. A higher priority has been the provision of a scientific publication in which the gradually unfolding Persian taxonomic discoveries can be communicated. In the past, most descriptions of new species, local floristic lists and ecological work have been

published in western journals; much taxonomic work has found its way into the *Notes of the Royal Botanical Garden, Edinburgh* for example. Now a new journal has been launched—the *Iranian Journal of Botany*—edited at the Ariamehr Botanical Garden in Tehran. It is evident from the first issue that this will be a most useful source of reference to all who are interested in the flora of the Middle East. Wendelbo provides an annotated check list of Iranian ferns; Eckblad summarised the scant information available concerning the gasteromycetes of Iran; biogeographical implications of newly described species are discussed and a floral list from the important Kavir Protected Region is included. The journal is in English and is lavishly illustrated with colour photographs; it is currently only available directly from the Ariamehr Botanical Gardens.

These efforts of the Iranians to distribute all available information must be highly commended. It must now be seen whether, as in the west, this dissemination results in the encouragement of further work. □

New discontinuity in the Atlantic

from M. Whitfield

ALTHOUGH, from the surface, the oceans may appear as remarkably homogeneous and featureless bodies of water they are in fact made up of an intricate three-dimensional patchwork of individual water masses. These water masses are layered according to their densities which in turn are defined by the salinity (S) and potential or *in situ* temperature (θ) of the component waters. The water masses are therefore most simply identified as clumps of points on a θ/S plot and the relationship between the nutrient (silicate, nitrate, phosphate) and oxygen concentrations and S or θ provide useful additional chemical fingerprints. One of the important tasks of physical and chemical oceanographers is to identify the origins of these bodies of water, to trace their movement through the oceans and to document their eventual decay by mixing with adjacent water masses. Such information is crucial to our understanding of oceanic mixing processes in general and in particular will affect our interpretation of the dispersion of man-made contaminants throughout the oceans.

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A recent paper by Broecker *et al.* (*Deep-Sea Res.* 23, 1083; 1976) presents the first results of an unusually detailed analysis of water masses throughout the Atlantic and illustrates some of the difficulties involved in unravelling these mixing patterns. They use results from the well-documented GEOSECS cruises which confirm many of the long established features of the Atlantic circulation pattern (Fig. 1). Their data indicate however that the boundary between the North Atlantic Deep Water (NADW, Fig. 1) and the Antarctic Bottom Water (AABW, Fig. 1), as indicated on θ/S plots, is far too sharp to be the result of vertical mixing between these two water bodies. They suggest that yet another water body is sandwiched between these two layers. This water is characterised by a potential temperature of 2°C (hence the name Two Degree Discontinuity Water, TDDW) and a salinity of 34.9‰ (Fig. 1). Water with these characteristics can be generated by mixing water from the North Atlantic, (Northern Component Water, NCW; 8 parts) with colder, less saline water from the Southern Atlantic (Southern Component Water, SCW; 1 part) which has a higher oxygen content and a lower silica content. TDDW can be traced throughout the Central Atlantic (from 35°N to 33°S) at depths from 4,000 m in the north to 3,200 m in the south. The concentrations of nutrients (phosphate, nitrate and silicate) and oxygen of TDDW in the western basins are consistent with the 8:1 mixing ratio. As the TDDW is traced towards the eastern basin the nutrient and oxygen patterns shift in a highly correlated



A hundred years ago

FINNISH papers report that vast masses of smoke are issuing from a mountain adjoining the river Tana, and that the snow in the vicinity has been melted away. The region has hitherto been free from evidences of volcanic activity. The theory has often been advanced that the gradual elevation of the shores of the Gulf of Bothnia is due to volcanic forces, and it is possible that these are finally seeking a vent.

The royal tigress in the Berlin Zoological Gardens, lately brought forth a litter of two, which she utterly refused to take care of. They were accordingly placed amidst the family of a Newfoundland dog, who welcomed the newcomers warmly and bestows upon them all necessary maternal attentions.

From *Nature* 16, 17 May, 54; 1877.

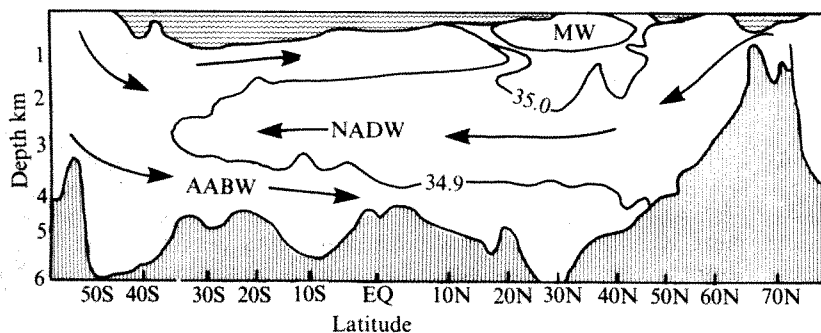


Fig. 1 Basic features of deep water movement in the Atlantic ocean as shown by a GEOSECS track down the centre of the western basin (adapted from Fig. 2 of Broecker *et al. op. cit.*). North Atlantic Deep Water (NADW) originating from the Labrador, Greenland and Norwegian Seas, flows south to split a northerly flow of Antarctic water. The Antarctic Bottom Water (AABW) results from the mixing of Weddell sea water and deep circumpolar water. A warm saline tongue of Mediterranean water (MW) intruding from the east interrupts the northerly flow of the upper layer of Antarctic water. Horizontal shading indicates the approximate extent of basal thermocline waters.

pattern that is consistent with the release of nutrients, originally locked up in biological detritus, by respiration and silica dissolution. It seems likely that upwelling areas off the west coast of Africa provide the main source of this organic debris so that nutrient levels rise and oxygen levels consequently fall on traversing from west to east.

Any hypothesis concerning the origin of TDDW must allow water masses from both ends of the Atlantic to be blended and spread over the whole central region. The rate of spread must be rapid enough to maintain the sharpness of the two degree discontinuity (TDD) against erosion by vertical mixing but not so rapid that the nutrient composition is homogenised throughout the layer. It is a measure of the difficulties inherent in understanding oceanic mixing processes that no convincing or consistent explanation can be given at this time for such a widespread feature.

A steady-state hypothesis proposed by Broecker *et al.* is based on the confinement of deep water movements by the Rio Grande Rise (at approximately 30°S) and suggests that TDDW is generated in the southern half of the Atlantic. This is inconsistent with the fact that the contribution of SCW to TDDW appears to decrease, rather than increase towards the south. It is also difficult to reconcile with observations that a young variety of TDDW (high in ¹⁴C and low in nutrients) is apparently forming just north of the 30°N boundary in the western basin. The alternative is to shed the concept of steady state mixing, which has so far formed the foundation of oceanic mixing models, and to assume that TDDW is a residual feature from an earlier stage in the ocean's history when the intrusion of AABW was much more extensive than it is now. The TDD, as we see it now, represents the cutting edge

of a more recent influx of NADW as it erodes away the remnants of this earlier intrusion from the south. Unfortunately the oxygen concentrations and the ¹⁴C ages of the various water bodies do not support the idea that water in the mixing zone is any older than the over-riding NADW. The mystery over the origin of TDDW is compounded by the fact that inflections in the θ and S against depth profiles are invariably several hundred metres deeper than the TDD in the western basin. This observation, which is inconsistent with the traditional physical picture of vertical mixing invoked in both the steady state and erosional models mentioned above, may provide a vital clue to the origin of this sharply defined and widespread feature.

Molecular genetics at Hirschhorn

by Eleanor Lawrence

The Third EMBO Annual Symposium, entitled Molecular Aspects of Gene Function, was held at Hirschhorn, West Germany, on 26-30 April 1977.

THE molecular biology of gene expression provided an umbrella large enough to shelter topics as diverse as bacterial chemotaxis, how the silkworm lays down its eggshell, and the structure of the cell's microfilament skeleton. But in the mainstream of molecular genetics the most rapid advance is undoubtedly in the dissection of the fine structure of the eukaryotic genome, thanks to the advent of recombinant DNA technology and fast sequencing methods. On the broader front, re-

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combinant DNA techniques promise to transform the study of the genetic basis of differentiation and development by bridging the gap between the biochemistry and classical genetic analysis.

At the level of the gene the hunt is now on for the elusive eukaryotic regulatory sequences. The complete sequence of the yeast 5S rRNA gene and its immediate flanking sequences has been determined by W. Gilbert and colleagues (Harvard University). Various interesting features appear in the region immediately preceding the transcribed portion but as yet the sequence alone can give few clues to the RNA polymerase recognition site, and there are no homologies with the well-known palindromic prokaryote promoter sequences. The gene is bounded at both ends by an oligo (dT-dA) tract. An oligo (dA-dT)-rich region has also been found by R. A. Flavell and A. Jeffreys (Amsterdam University) in the 5' region flanking the rabbit α -globin gene. Using globin cDNA plasmids as probes to identify globin gene sequences in rabbit DNA, they have been able to construct a restriction map of the chromosomal gene and its surrounding regions and have subjected these to preliminary analysis.

In the sea urchin histone repeat unit being sequenced by M. Birnstiel and his colleagues (University of Zurich), a partially homologous sequence is found 20 nucleotides upstream of both the histone H1 and H2A mRNA initiation sites, and another homology occurs some 100 nucleotides upstream in both genes. To determine what part if any these sequences play in gene expression Birnstiel intends to use the oocyte transcription/translation system described by John Gurdon (MRC Laboratory of Molecular Biology, Cambridge) (see *Proc. natn. Acad. Sci. U.S.A.* 74, 1502: 1977). In principle, the promoter region could be identified by producing various DNAs containing the gene plus different lengths of flanking sequence and determining which of these are transcribed when injected into the oocyte nucleus. Birnstiel has also made some modifications to the technique which he claims make it easier to use by those inexperienced in oocyte nucleus injection—centrifugation makes the nucleus bulge out the oocyte wall leaving a neat ring of pigment granules as an injection target.

Nucleotide sequences alone have failed to betray the eukaryotic promoters; but novel and exciting techniques of DNA modification (Gilbert) can be used to determine the exact binding position of protein on DNA if the nucleotide sequence is known and may provide a direct handle on the problem. Using the phosphate-specific

reagent ethylnitrosourea, Gilbert has shown that in the *lac* repressor-operator model system the *lac* repressor binds to one side only of the DNA double helix rather than being wrapped around the DNA. The bases involved in RNA polymerase recognition are also being investigated, by examining the effects of RNA polymerase binding to *lac* DNA on the reactivity of the individual bases to modifying reagents.

Another approach to the analysis of *lac* operator-repressor binding was demonstrated by J. H. Miller (University of Geneva). He and his colleagues have used novel genetic techniques (see *J. molec. Biol.* **109**, 275, 303; 1977) to generate repressors with known amino acid changes. Among them is one with almost 10,000 times wild type affinity for operator and which also binds to non-operator DNA such as lambda and SV40. By determining whether this non-specific binding involves any particular sequences they hope to identify those features of the binding sequence which are common to all and thus identify the parts of the operator crucial for repressor recognition. Using the nonsense mutations accumulated during this work, they have also been able to determine the characteristics of one type of mutational 'hotspot'—genetic regions which are much more easily mutated by specific mutagens. They find that the hotspots preferentially mutated by 2-aminopurine and at which mutations also arise spontaneously are always part of a unique sequence which has 5-methylated cytosines in a particular position (the *i* gene has been sequenced by Gilbert and colleagues). This tie-up between methylated bases and mutability leads Miller to speculate on the wider role of methylation *in vivo*.

In connection with recognition sites on mRNA several speakers, especially L. Gold (University of Texas) reminded participants that the three-dimensional structure must be considered and not just the sequence. He illustrated this with reference to a theoretical structure for *lac* repressor mRNA.

Any discussion of eukaryotic gene expression however, has to take into account that in reality eukaryotic DNA is packaged into chromosomes in the form of chromatin. *In vitro* transcription of chromatin provides one way of investigating the possible regulatory role of the associated histone and non-histone proteins. B. O'Malley (University of Houston) reported a set of experiments on transcription of chick oviduct chromatin. Using *E. coli* RNA polymerase, and a plasmid-derived probe of ovalbumin cDNA, he showed that oestrogen-stimulated oviduct chromatin retained transcriptional specificity *in vitro*, and by reconstitution

experiments that this specificity is mediated by non-histone proteins. Although these results are not novel (Paul and Gilmour published similar data several years ago as did other groups) the low level of endogenous sequence, combined with the use of mercurated nucleotide precursors to allow isolation of newly-synthesised RNA, make this work the most convincing presented to date. However, as P. Chambon (Institut de Chimie Biologique, Strasbourg) stated in discussion this is still a controversial area of research and the proponents of chromatin reconstitution do occasionally shift their arguments to meet the latest technical advances. Perhaps the most impressive result O'Malley presented was that he could switch on ovalbumin mRNA transcription by adding the progesterone/receptor complex to isolated 'switched-off' chromatin; this natural hormone switch activated the chromatin some 10 to 50 times over the control. O'Malley is now isolating genome DNA sequences containing the ovalbumin gene by affinity chromatography on cDNA and mRNA cellulose columns, and has already obtained a purification of more than 100,000-fold. Soon he will have sufficiently pure DNA to clone the gene sequences and so be able to study the control regions near the structural gene.

The search for possible gene-specific or sequence-specific DNA-binding proteins which may play a part in regulating the expression of particular genes was illustrated in work reported from W. Gehring's laboratory (Biozentrum, Basle). Two DNA-binding proteins isolated from unfertilised *Drosophila* eggs were shown to bind specifically to two chromosomal fragments from Gehring's bank of cloned DNA fragments representing the complete *Drosophila* genome. One of these hybridises *in situ* only to the nucleolus, strongly suggesting that its corresponding DNA-binding protein is specific for ribosomal DNA sequences; the other hybridises to one band on chromosome 3.

The genetic basis of differentiation and development is now under investigation in a variety of systems. One of the most promising is that described by F. Kafatos (University of Athens) in the silkworm *Bombyx*. Its chorionic proteins which are laid down in strict sequential patterns to form the eggshell, are determined by a system of two or three multigene families whose mechanisms of regulation and expression during differentiation and development are now, with recombinant DNA technology, amenable to full analysis. *Bombyx* also obligingly provides several chorionic mutants for comparative analysis.

After determining the protein electrophoretic pattern at different stages of eggshell formation, Kafatos together with T. Maniatis and colleagues at Harvard University is now constructing cDNA plasmids representing specific chorionic mRNAs, from the heterogeneous mRNA population present during choriogenesis. Some 20 different clones have been identified so far, two of which have been found to represent proteins expressed at the same developmental stage and one at a different stage. By looking at the pulse-labelled nuclear RNA at various stages using the cDNA plasmids as probes, it will be possible to answer the question of whether the protein pattern is controlled by specific transcription or by translation of pre-existing messengers. Chromosomal DNA clones are also being constructed and will be used to determine whether evolutionarily similar chorionic protein genes are linked, to determine whether genes expressed at the same time are linked, and to study the putative regulatory sequences flanking the genes.

One interesting report (arising out of the study of the developmental genetics of the slime moulds) was R. A. Firtel's (University of California, La Jolla) description of translation of a cloned actin gene from *Dictyostelium* in *E. coli* minicells. So far his evidence consists of the appearance of a new protein, which runs in the same position as actin on one-dimensional gel electrophoresis. Confirmation of specific translation awaits the full identification of this new protein.

Now that it has become possible to look directly at the regulatory sequences surrounding the gene a great deal of attention is being focused on the transcriptional control of gene expression. But regulation also occurs at many other levels. Differential stability of mRNAs as well as differential transcription must be invoked to explain the patterns of mRNA abundance and copy complexity found in some developing systems, and R. Williamson (St Mary's Hospital Medical School, London) reviewed evidence that control is also exerted at the stage of mRNA processing from nucleus to cytoplasm. J. Bishop (University of Edinburgh) reported evidence from his laboratory that in different types of mouse cell a substantial proportion of the total mRNA sequences are common to the different cell types at a low level. Margaret Buckingham (Institut Pasteur, Paris) described changes in mRNA copy complexity and abundance in myoblast differentiation and also showed that particular mRNAs are more stable in differentiated myotube compared with myoblast cells, whereas others show no change. □

review article

Super-luminal expansion in extragalactic radio sources

R. D. Blandford*, C. F. McKee† & M. J. Rees‡

Very long baseline (VLB) investigations of compact extragalactic radio sources can often be interpreted in terms of two non-thermal components apparently separating with speeds in excess of the speed of light. Given the conventional physical laws and the cosmological interpretation of large redshifts, the available morphological evidence, while inconclusive, is most compatible with a general class of models in which a signal propagating with a speed close to that of light is scattered by an appropriately shaped screen. Possible observations are discussed which might discriminate between this and alternative explanations.

THE use of very long baseline (VLB) interferometry has made it possible to resolve hitherto inaccessible details of compact extragalactic radio sources, and seems to show that they are made up of two or more components. The original observations have now been repeated at intervals over the past five years, and have revealed that these components are in the process of moving apart. The spectacular implication of this discovery is that, if the radio sources are at the large cosmological distances suggested by their red shifts, the components must be separating at apparent transverse velocities more than twice the speed of light¹⁻³. In this article we review and assess the various theoretical explanations that have been proposed to account for this remarkable phenomenon, our main aim being to emphasise the theoretical and observational uncertainties which must be resolved before these objects can be reliably interpreted.

The projected sizes of the rapidly expanding components are typically only a few parsecs and so they are presumably linked to the primary energy source powering the associated active galactic nucleus—indeed this is one reason why VLB observations are of such great astrophysical interest. However, observations in other parts of the electromagnetic spectrum have sometimes shown even more rapid variations^{4,5}; light travel time arguments imply that the 'prime mover' may be even smaller than the radio source.

We have attempted to provide a loose kinematic classification for the models discussed in this paper as shown in Fig. 1 (although the boundaries between the different categories are rather blurred and the classification is not exhaustive). In 'intrinsic' models, the apparent expansion is directly related to the radio emission process; these models are 'causal' or 'acausal' depending on whether or not the expansion is due to one or more independent events. In 'extrinsic' models, the apparent expansion results from scattering or focussing by the intervening medium along the line of sight.

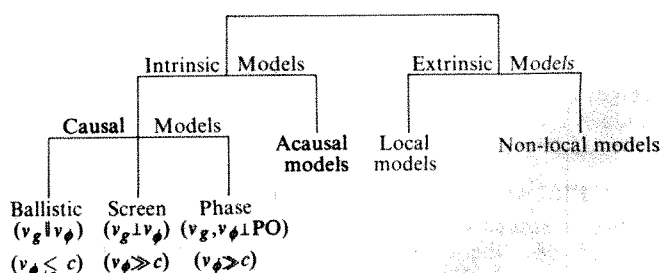
The radio emission is commonly attributed to an incoherent synchrotron-like process involving relativistic electrons (although the evidence for this is less convincing than for extended radio sources). Many of these sources have had their total

flux monitored^{6,7} for more than ten years and have long been known to be variable. In many cases, the synchrotron hypothesis can lead to a theoretical difficulty, the so-called 'inverse Compton catastrophe'^{8,9}. This can arise if the linear size of the source is limited to the distance light can travel in the time taken for the flux density to halve, thus putting a lower limit on the radiation brightness temperature and energy density and an upper limit on the magnetic energy density. When the dominant contribution to the energy density is in the form of radiation rather than magnetic field, the total energy requirements of the source then become implausibly high. This problem disappears if the sources are expanding with speeds $\sim c$ (refs 9–12). Furthermore, in most sources, the relativistic particle pressure is found to exceed the magnetic pressure⁹. Therefore, unless the relativistic plasma is bound by a much higher density of thermal plasma (and this can probably be excluded¹³ on other grounds), relativistic expansion is to be expected. So these arguments, which are independent of the observed angular structure (and were given before the VLB observations), at least support models involving intrinsic relativistic expansion which as we shall see seem most attractive on other grounds.

Dynamics of relativistic expansion

Our understanding of relativistic gas dynamics is largely derived from solutions of a few idealised problems. We mention some of these here in order to demonstrate that relativistic kinematical effects (discussed below) can be accommodated in a framework that is both dynamically and astrophysically plausible.

Fig. 1 Possible classification of the physical models discussed in the text.



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We shall consider first the free expansion of an initially uniform spherical cloud of relativistic gas. If an initially uniform cloud of ultra-relativistic plasma starts expanding from radius r_0 , then the Lorentz factor of the bulk expansion when the sphere attains a radius $r \gg r_0$ would be $\gamma \sim r/r_0$ (refs. 14–16), the material being concentrated mainly in a thin shell of thickness $\sim r_0$. In order to conserve total energy, the internal (thermal) energy per particle, measured in a co-moving frame, must be reduced by a factor $\sim r_0/r$. If the cloud is composed of electrons and protons, then the bulk Lorentz factor will saturate when the energy per particle $\sim m_p c^2$ and the characteristic electron energies will subsequently decay $\propto r^{-2/3}$ if they expand independently from the protons with a specific heat ratio of 4/3. In some models in which the central energy source derives from 'magnetic accretion' on to a Kerr black hole, an electron-positron plasma may be produced¹⁷ and this could attain bulk Lorentz factors comparable with the inferred initial random Lorentz factors of the emitting electrons. Free expansion can occur either impulsively, if the surrounding medium is dynamically unimportant, or continuously in a supersonic wind. If this wind were initially subsonic, then either a nozzle geometry¹⁸ or a relativistically deep potential well¹⁹ would be needed to achieve a steady relativistic trans-sonic change. Variability could be induced in an expanding wind by changing the volume emissivity in a dynamically insignificant fashion. However, if the emission processes are synchrotron and inverse Compton radiation, the observed optically thin power will typically decay very rapidly with radius and so it should be dominated by the model-dependent conditions at small radii²⁰.

Second, we consider relativistic blast waves. If the external medium has a density $\rho > E/r^3 c^2 \gamma^2$, with E the energy of the expansion, then it cannot be ignored. A strong shock front will expand outwards with a Lorentz factor $\sim \sqrt{2\gamma}$. The swept up material will be concentrated within a shell of thickness $\sim r/\gamma^2$ and if the explosion is impulsive with the external medium uniform, $\gamma \sim (E/\rho c^2 r^3)^{1/2}$ (refs. 20–22). When r gets so large that the shock stops being relativistic then there is a smooth transition towards the ordinary Sedov²³ solution. Any realistic dynamics compatible with the VLB data must certainly involve deviations from spherical symmetry. Fortunately these results are easily generalised to non-spherical geometries as long as the expansions are at least mildly relativistic. This is because, to a first approximation, each segment of the expanding shell moves independently. A dynamical advantage of blast wave models is that the shocks can be radiative, so that the mechanical energy associated with the explosion can be transformed to radio emission with a high efficiency. This is in contrast with free or adiabatic expansions and models in which the radio emission is associated with a moving plasmoid.

Finally, we may envisage a fast-moving condensed object. The radio-emitting plasma need not be undergoing any expansion if it is coupled (either electromagnetically or by ram pressure) to a rapidly moving object. One attractive conjecture is that when a binary system comprising two condensed objects (neutron star or black hole) coalesce as a result of the radiation of gravitational waves, tidal effects become important and the individual components are broken up²⁴. One or more components of appreciable size can then be ejected relativistically²⁵. Note that in this case the expansion speeds will be constant, instead of accelerating or decelerating as in the case of free expansion of relativistic gas or relativistic blast waves.

Of course, some aspects of all three possibilities may be involved. For instance, the early phases of an outburst may resemble freely accelerating expansion, followed in the later stages by either gradual deceleration in a uniform medium or sudden retardation when a comparatively dense phase of the surrounding material is encountered.

Kinematics of causal models

When a source changes in a time comparable with its size in units of c , the kinematics of the emitted radiation must be carefully analysed and it is possible for a distant observer to

see apparent super-luminal expansion. For causal models, we imagine that an outburst occurs at a point P at a time $t = 0$, as measured by a distant observer O. A 'signal' such as a blast wave^{20,22} or a burst of low frequency radiation from a newly-formed pulsar^{26–28} then moves radially outwards from P. This signal may either be directly associated with the emission (for example, in the case of a radio-emitting plasmoid)^{10,11} or trigger it (for example, in the case of a low frequency electromagnetic wave that can accelerate electrons but not ions to relativistic energies, so that the electrons are held in place by the inertia of the ions)²⁷. We distinguish four speeds associated with this signal: a group velocity $v_g < c$ which is the material velocity of the signal; a phase velocity v_ϕ which is the space velocity of some directly observed feature such as a radio centroid or the outer edge of a resolved component; v_0 , the apparent velocity observed at O, normal to OP; and the actual physical velocity $v_{em} < c$ of the emitting material or of the frame in which the emissivity is approximately isotropic). In some models all four speeds are different. v_0 and v_ϕ are related by

$$v_0 = \frac{v_\phi \sin \theta}{1 - (v_\phi/c) \cos \theta} \quad (1)$$

or

$$\cos \theta_{\pm} = \frac{v_0^2/c \pm [v_\phi^2 + (v_\phi^2 v_0^2/c^2) - v_0^2]^{\frac{1}{2}}}{v_\phi [1 + (v_0/c)^2]} \quad (2)$$

where θ is the angle between v_ϕ and PO.

In 'ballistic models' of super-luminal expansion, the phase and group velocities are both approximately parallel to OP and usually approximately equal. From equation (1) we then see that $v_0 \leq v_\phi \gamma_\phi$, where equality implies that $\theta = \cot^{-1}(c/v_0)$ large observed speeds v_0 can only occur if v_ϕ , which is generally $< c$, is almost parallel to the line of sight. As an example, consider a spherical model in which $v_g = v_\phi$ is the relativistic radial velocity of a radio emitting shell. If v_g is constant, then the surface observed at a fixed observer time t is an ellipsoid with focus at P and eccentricity v_g/c ^{10,11,20,29,30}. The observed width is the minor axis of the ellipsoid, $2\gamma_g v_g t$, ($\gamma_g = (1 - v_g^2/c^2)^{-1/2}$), and so the observed expansion speed is $v_0 = 2\gamma_g v_g$ which exceeds $2c$ when $v_g > 2^{-1}c$. The observed shape can be straightforwardly calculated when v_g is a function of position. If, for instance, v_g changes only with radius r the locus becomes an ovoid, the blunt end being towards O for a decelerating (and the sharp end for an accelerating) expansion. Provided that $d \ln \gamma_g / d \ln r = -n < \frac{1}{2}$, $v_0 = 2\gamma_g v_g \propto t(-n/(2n+1))$, where v_g is the group velocity of the edge of the observed disk at the retarded time. If the emitting region of the source is enlarging in this fashion then it follows that the actual source dimensions are $\sim \gamma_g$ larger than the projected size (that is, $\sim \gamma_g^2 ct$), assuming approximate spherical symmetry.

Clearly a spherically symmetric model cannot explain double structure. In a straightforward modification, two radio-emitting plasmoids are ejected along anti-parallel directions^{31,32}, the velocity of the nearer making a small angle $\sim \gamma_g^{-1}$ with PO, the super-luminal velocity being mostly attributable to the approaching plasmoid. However, this occurs with a probability $\sim (\cos \theta_+ - \cos \theta_-) \leq 2c^2/v_0^2$, which is typically very small when $v_0 \gg c$.

In 'screen' models, v_ϕ and v_g are approximately perpendicular and the radio emission is triggered by a relativistic signal ($v_g \sim c$) illuminating a pre-existing surface so that $v_\phi \gtrsim c$. Usually v_ϕ is normal to the line of sight so that $v_0 \sim v_\phi$. A possible physical manifestation is the intersection of a relativistic blast wave with a dense plasma (or another blast wave) wherein the kinetic energy can be efficiently radiated away. Of course, with an appropriately designed screen, we have $v_0 \gg c$ (for example, if a relativistic signal illuminates a paraboloidal screen with focus P, axis PO). For a roughly spherical screen of radius R illuminated by a concentric spherical pulse of speed c , a distant observer will see a circle of radius $\sim (2Rct)^{\frac{1}{2}}$

for $t \ll R/c$ that seems to expand super-luminally until the apparent radius $\sim 2^{-1}R$ (refs 33, 34). If the screen is a thin ring (for example, a rotating, gaseous disk surrounding a central gravitating object), then the observer will see an expanding double whose separation is decelerating. (Deceleration is characteristic of screen models and is generally more likely to occur than acceleration.) A rotating searchlight³⁴ forms the basis for a kinematically similar model.

The signal motion need not be rectilinear. One ingenious suggestion³⁵⁻³⁷ is that the emitting electrons are channelled at relativistic speeds along the north and south polar field lines of a magnetic dipole. They would then be visible only when they intersected the line formed by the locus of field lines tangent to OP. If the emitting particles are all generated in a single outburst then an expanding double ($v_0 = 4.4c$ (ref. 35) in the equatorial plane) will be seen aligned with the rotation axis. However, because the synchrotron emissivity falls off so rapidly ($\propto r^{-9}$ if the magnetic moment is preserved), it is difficult to account for the equality of the radio components or to evade prohibitive synchrotron losses at early times in this model.

In most types of simple screen model super-luminal effects can usually be achieved without the observer's having a privileged orientation (in contrast to ballistic models). Apparent velocities $v_0 \gg c$ will, however, only be observed for a fraction $\sim (c/v_0)^2$ of the total emission time (the duty cycle) and the actual size of the screen will typically exceed the observed size by a factor $\sim (v_0/c)$. In 'oriented' screen models, the screen is either localised along the line of sight or a large section of it is almost tangent to one of the retarded ovoids. In this case the observed size can be comparable with the actual size and the duty cycle increased to about unity, but only at the expense of requiring the observer to have a particular orientation with respect to the screen [with probability $\sim (c/v_0)^2$].

One can perform similar calculations for more complex geometries. The results obviously depend sensitively on the shape and orientation of the screen and somewhat on v_s . (Note that in the special cases of the sphere and the ring, the kinematics is independent of v_s .)

In Fig. 2 we display the results of a calculation of the observed shape when a spherical pulse moving with a speed $v_s \sim c$ illuminates an axisymmetric screen tailored to produce an expanding double. In this model, separation speeds $\lesssim 5c$ can be seen with most observer orientations.

The third type of causal explanation, which we term a 'phase' model, arises when v_0 and v_s are mutually parallel, and approximately perpendicular to the line of sight. This configuration occurs when, for instance, a synchrotron source is assumed to trail behind a moving object^{38,39}, expanding transversely to the direction of motion. The point of maximum brightness can move with $v_0 \gg c$ even when $v_s \ll c$; and, in a suitable geometry, an expanding double source can be produced. However, in order that the regions of highest brightness separate with an observed velocity $v_0 \gg c$, it is necessary that the object move along a line lying almost in the plane of the sky, so that $\cos\theta_{\pm} \sim (c/v_0)(1 \pm v_0/v_s)$. This will occur with a probability $\frac{1}{2}(\cos\theta_+ - \cos\theta_-) \sim c/v_0$ (using equation (2)). For orientations outside this range, light travel time effects will reduce and may even reverse the observed velocity. Again this is a serious problem for this type of model as it either requires the sources to be in a super-luminal mode for a small fraction of their life, or that most expansions be non-relativistic. However, it does have an advantage over ballistic and screen models in that the actual source size can be comparable with what is observed ($\sim v_0 t$).

Acausal models

Changes in VLB structure need not, however, be attributed to a single agency. For instance, 'Christmas tree' models^{40,41} have been proposed in which independent sources (lying in a disk, viewed approximately equatorially) light up successively so as to

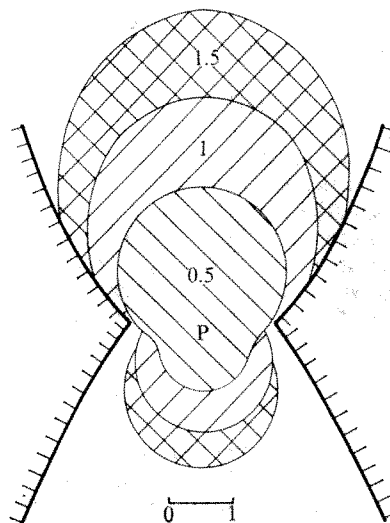


Fig. 2 Specific example of a 'screen' model. A central 'powerhouse' P, such as an accreting black hole or a compact cluster of pulsars), is presumed to be responsible for the production and maintenance of two anti-parallel beams of relativistic fluid. The walls of these beams are taken to be confocal paraboloids of unit semi latus-rectum. A shock with speed $\sim c$ travels radially outwards from P and radio emission is presumed to occur after this shock hits the channel walls for a duration of $0.5/c$. The observer is inclined to the symmetry axis with the most probable inclination of 60° . The emitting material is pushed radially outward with a mild relativistic speed ($\sim c/3$) so that the radio emission is bright only from those parts of the wall where the radius vector makes an angle $\lesssim 80^\circ$ (corresponding to $\mathcal{D} \sim 1$) to the observer direction. The diagram shows the shapes observed at times $t = 0.5/c, 1/c, 1.5/c$. VLB observations of this outburst with a linear resolution ~ 2 would probably be interpreted in terms of an expanding double source in which the separation speed decelerates from $\sim 5c$ to $\sim 4c$. The flux from the two sources would be comparable, the reduced area in the lower component being somewhat compensated by an increased surface brightness resulting from it being closer to P. Of course, the particular details of this model are *ad hoc*, but it does demonstrate that a super-luminally expanding double source can arise quite naturally in the twin beam geometry. Separation speeds $\sim 10c$ can be achieved if the orientation has a less probable value $\lesssim 30^\circ$, but in this case the observed brightness distribution will probably be less symmetrical and the variation with time more dramatic.

mimic a regular expansion. One objection frequently raised to these models is that only expansions, rather than equal mixtures of expansions and contractions, are generally observed. However, as an example, if there are three stationary radio sources aligned so that they are not quite resolved and the central component flares irregularly with a rise time much shorter than the decay time, whilst the outer components have the time-reversed behaviour, then apparent expansions will usually be observed³⁸. In not all sources is the resolution adequate to exclude this type of model.

Doppler effect

The foregoing discussion has been concerned primarily with the rate of expansion of the projected source dimensions and involved only the source geometry and the effective value of γ_s . The relative surface brightness of different parts of the source depends on various astrophysical effects—particle acceleration, magnetic field strength, energy and isotropy of the hypothetical central outburst and so on, of which our knowledge must be limited. However, one important effect which we can consider is the Doppler shift. Its parameters are given by the factor $\mathcal{D} = [\gamma_{em}(1 - (v_{em}/c) \cos\theta)]^{-1}$ where θ is the angle between the velocity v_{em} of the emitting material and OP. The intensity from a given element of proper

volume is then $\propto \mathcal{D}^{3+\alpha}$ (ref. 42) where α is the spectral index. When the material responsible for the emission shares the group velocity, that is, $\gamma_g \sim \gamma_{em}$, then $\mathcal{D}$ ranges from $\gamma_g(1+v_g/c)$ to $[\gamma_g(1+v_g/c)]^{-1}$ for motion parallel and anti-parallel to OP. On the other hand, if there is no bulk motion and v_g is, for example, the group velocity of a low frequency wave pulse, then there is no Doppler shift. We would argue that the true situation lies between these two extremes which are both difficult to reconcile with the observations.

For ballistic models in which $\gamma_g \sim \gamma_{em} \sim \gamma_0$, those parts of the source which display the largest superlight velocities would all have $\mathcal{D} \sim \gamma_{em}$ and this enhances the probability of observing the effect. Indeed one can even envisage a 'shrapnel' model¹⁰ in which $\sim \gamma_{em}^2$ sources are ejected in random directions but the two with $\theta \lesssim \gamma_{em}^{-1}$ are so strongly highlighted that only an expanding double (with, however, a randomly oriented axis) is seen. On the other hand, one cannot plausibly interpret a super-luminal expanding double as two plasmoids moving in opposite directions unless their proper emissivities decay coincidentally as $\tau^{-(3+\alpha)}$ (ref. 43) where τ is the proper time. (If this were the case then the total flux density should fade $\propto d^{-(3+\alpha)}$ where d is the component separation, a possibility that can already be excluded for 3C273.)

The hypothesis that $\gamma_g \sim \gamma_{em}$ then runs into difficulties because it would give rise to a very non-uniform surface brightness making it particularly difficult to account for equal doubles aligned along a preferred axis. However, if $v_{em} \ll c$ and the Doppler effect does not apply, as can occur in both screen and ballistic models, super-luminal expansion will last only a small fraction $\sim (c/v_0)^2$ of the light travel time across the emitting region. So if one averages over a time interval larger than the light travel time, then only $\sim (c/v_0)^2$ of the flux reaching the observer would be associated with an apparent speed v_0 . Moreover, this applies at every instant if, as seems to be the case for 3C120 (ref. 44), the outbursts seem to overlap. Present observations indicate that the flux from the high speed components is not dominated by a slowly changing background so that some bulk relativistic motion of the emitting material must occur. Note that even a velocity of $v_{em} \sim c/3$ would give brightness varying by a factor $2^{3+\alpha}$ as θ increases from 0 to π . In most sources, the source brightness temperatures directly measured by VLBI are $\lesssim 10^{12}$ K. This implies that only mildly relativistic¹² ($\gamma_{em} \lesssim 2$) bulk Lorentz factors are necessary to evade the self-absorption and inverse Compton constraints in incoherent synchrotron emission models. This is consistent with a model such as that illustrated in Fig. 2 in which the kinematics is dominated by the shape of the screen rather than by bulk motion.

In phase models displaying super-luminal expansion $\theta \sim \pi/2$ and the variation in $\mathcal{D}$ across the source is unlikely to be important even if $v_{em} \sim c$.

Propagation effects in extrinsic models

In extrinsic models, the rapid expansions are attributed to radio propagation effects: a small source switches on, the first radio emission to reach the observer follows a direct route and the apparent expansion results from radiation which is focused or scattered towards the observer after traversing increasingly curved paths with consequent progressively larger time delays. If the radiation is bent through a small angle ϕ while propagating out to a distance r , the apparent size would be $\sim 2\phi r$ and the expansion velocity $v_0 \sim 2c/\phi$. The objection to isotropically scattering stationary screens—that the flux be dominated by back scattered radiation—does not apply in this case as long as $\phi \ll 1$.

In 'local' models, processes operating on scales of tens of parsecs must scatter, reflect, or refract radio wave through angles of a few degrees. However, this can usually only be done at the expense of restricting the size of the central source

of radio waves, assumed to be roughly spherical, to a fraction $\sim c/2v_0$ of the observed size, thus increasing the brightness temperature and exacerbating the 'inverse Compton catastrophe'. By contrast in intrinsic source models the observed source size is typically a lower bound on the total linear dimension.

One specific mechanism that could operate in this way, and is probably at least a complicating factor in other models, is induced Compton scattering⁴⁵. If the brightness temperature of the central source is $T_b > m_e c^2/k \sim 10^{10}$ K, then a surrounding cloud of non-relativistic plasma would have an optical depth to induced scattering of $\tau_{ind} \sim (kT_b/m_e c^2)\phi^4 \tau_{spont}$. (ref. 46) where τ_{spont} is the optical depth to spontaneous Thompson scattering. If $\tau_{ind} \gtrsim 1$ while $\tau_{spont} \ll 1$, only induced scattering will be important and this is restricted to those directions that are already occupied by photons. So in such a model there would have to be a background source of angular size comparable with the maximum observed angular separation the brightness distribution from which would be supplemented by photons contributed by the rapidly fluctuating component. However, as radio brightness temperatures do not much exceed $\sim 10^{12}$ K, induced scattering cannot explain expansion speeds $v_0 \gtrsim 6c$ without invoking a specialised geometry. It also seems difficult to produce an equal double by this mechanism unless the observer happens to be located close to the equatorial plane. Nevertheless, induced scattering does involve a systematic lowering of a photon's frequency, and so a general softening of the radio spectrum is expected as the outburst develops⁴⁵, as is indeed frequently observed. (In extreme conditions, a small increase in radio brightness temperature can even occur).

In an alternative scattering mechanism, radio photons of wavelength λ can be deflected through an angle $\delta\phi \sim \lambda/\lambda_p$ by scattering a longitudinal plasmon of wavelength λ_p (ref. 47). If the turbulent energy density in the plasma surrounding the radio source can be maintained at a level sufficient to scatter each radio photon through $\sim 10^\circ$ then again an apparent expansion will be observed following an impulsive outburst. However, as the scattering cross section per electron $\propto \lambda^2$, the effect is strongly frequency-dependent. When the plasma turbulence is approximately isotropic, the maximum scattering angle $\phi \propto \lambda^2$; and when the plasmons are unidirectional, as they would be if they had a discrete origin separate from the radio photons, then $\phi \propto \lambda^3$. So on these models if most of the radio flux is resolved into an expanding source with speed v_0 then the effect should largely disappear if the frequency is reduced by factors $\sim (c/v_0)^{1/2}$, $\sim (c/v_0)^{1/3}$ respectively, as the observed emission will then be dominated by large angle scatterings from earlier outbursts. Provided that the plasmon phase velocities $\ll c$, the frequency shift associated with these scatterings will be negligible and so the spectral variation in an outburst would have to be intrinsic to the source.

A third possibility is the small angle refraction of radio waves by a surrounding cloud of dense ionised filaments, possibly associated with optical emission lines. (In order to produce an expanding double aligned with the symmetry axis, either the individual filaments must be elongated in the equatorial direction or the distribution of scatterers would have to be extended along the axis.) Scattering angles $\sim 10^\circ$ can be plausibly achieved at cm wavelengths using typical emission line region parameters, but again as the effect is $\propto \lambda^2$ any prominent angular structure should disappear on trebling the wavelength. The available evidence does not support this prediction, which would also include strong deceleration.

A somewhat different local effect might be the obscuration of part of a spherical source by an equatorial disk of free-free absorbing plasma. In this way a closely separate double can be produced. However, in order to see a widely separated equal double, the observer must lie in the equatorial plane.

A possible non-local effect is the operation of a gravitational lens^{48,49}. If a compact mass lies along the line of sight it yields a 'double crescent' image. An angular scale of milli-arc seconds

requires a lens mass $\sim 10^5 M_\odot$. A lens model has the virtue that the components are generally of comparable flux density whenever the magnification is high. However, it does not seem possible to achieve systematic expansion by more than the unmagnified source size, and there would be no reason for expecting compact doubles produced in this manner to display any preferential alignment with the axis of an associated double source. An alternative non-local effect involves intergalactic scintillation associated with fluctuations in the density of plasma in the intergalactic medium or an intervening galaxy⁵⁰. This effect would operate similarly to local scintillation and again would be expected to be strongly frequency dependent.

Observational tests

We have analysed the most important features of several different models designed to explain the prime observational fact of extragalactic VLBI—the prevalence of compact double radio sources expanding super-luminally along a preferred direction. In fact, the observational evidence for this property is derived as much for composite studies of several sources as from detailed investigations of individual nuclei and so must still be treated with considerable caution. Nevertheless, improvements in techniques and processing capacity within the next few years should clarify the picture. On the basis of this feature, we have argued that the most attractive theoretical explanations (that is, those least incompatible with existing observations), involve the illumination of an appropriately shaped screen (for example, the cylindrical walls of two anti-parallel relativistic beams) by a 'signal' moving at the speed of light (for example, a relativistic blast wave or a pulse of low frequency radiation), originating from a more compact central source (which might be a $10^8 M_\odot$ black hole). In these models it seems necessary that the emitting region move outwards with a mildly relativistic speed. The arguments advanced against alternative 'intrinsic' models are mainly probabilistic; and most 'extrinsic' models be ruled out because they predict an apparently unobserved frequency dependence. These arguments too must be treated with scepticism at this stage. We conclude by listing some additional observational tests and outlining the conclusions that it may be possible to draw from them.

(1) Do systematic source motions always correspond to expansions rather than contractions? If so it would form the strongest evidence against naive acausal 'Christmas tree' models. It would be of particular interest to know to what extent successive expansions overlap in time and what fraction of the total compact component flux is associated with moving components, as this would indicate how rapidly the emitting region would have to recover so as to accommodate the next outburst, and how rapidly it would have to expand so that only the front of the source would be observed.

(2) Does the position angle and/or the expansion speed vary during a single outburst? Models involving relativistic bulk motions can produce either accelerating or decelerating expansions. Indeed for those sources where the component separation is observed to expand by a factor ~ 2 , it would be surprising if some change in the observed velocity were not seen. Blast wave models are generally decelerating, whereas free expansions, which are perhaps unlikely in view of the rapid decline with time of their optically thin emission, may accelerate. The expansion rates for screen models are determined mainly by the shape and orientation of the screen, but if the outburst or screen are not axisymmetric, the position angle may change⁵³. One interesting possibility is that the velocity be accurately constant during an outburst. In this case the expansion would probably have to be attributed to one or more coherent objects moving inertially, although in certain conditions blast wave models can exhibit constant velocity phases²¹.

(3) Are the position angle and expansion speed 'remembered' from one outburst to the next? Successive outbursts should

display the same alignment if they represent expansion along a preferred direction (for example, the rotation axis of a central massive object or rotating gas cloud). A reproducible expansion speed would be a strong argument in favour of a screen model, but the absence of such would not necessarily rule it out because the signal velocity v_s could vary between outbursts, which would alter the kinematics.

(4) What are the absolute positions of the components and which ones are moving fastest? Clearly the development of phase stable VLBI would require a considerable technical advance. However, it may be possible to learn which parts of the source are moving if either the nucleus can be resolved into two or more components, one of which is unchanging, or, in the case of triples, if it is assumed that the central component is stationary. Alternatively, for those sources for which there is a sufficiently nearby unresolved radio source (for example, 3C345/NRA0512), it may be possible to obtain some absolute positional information.

(5) How do the components of expanding doubles evolve? If the emission is due to synchrotron radiation, the component size must always be large enough to bring the brightness temperature below the plausible upper limit set by the inverse Compton restriction. It would be important to know how the source size or surface brightness distribution depends on wavelength, and also whether or not a particular component faded out earlier and was a greater fraction of the flux at short as opposed to long wavelengths, as this would be a particular signature of propagation effects. Other models make specific predictions about wavelength dependence³⁹. Some models also make rough predictions about the distribution of surface brightness^{30,51}. For instance, in the twin-beam model^{18,52} illustrated in the figure, we might expect source asymmetry to increase with the apparent velocity. In simple screen models, the faster component would have lower brightness, whereas in models where the Doppler effect is more important, the reverse would be true. In sources showing successive outbursts, higher sensitivity observations would allow an old and fading component to be followed in the presence of a young and bright source. This could considerably improve the interpretation of the kinematics.

(6) What are the polarisation properties of the individual components? If as proposed on some models the emitting electrons are channelled along an effectively rigid magnetic field, then the observed radiation should be strongly polarised normal to the source axis if it is synchrotron radiation³⁶, and parallel if it is curvature radiation³⁷. If the field direction makes a small angle to the line of sight then perhaps a substantial circular polarisation⁵³ could be observed (unless electrons and positrons are created in equal quantities). The induced Compton and plasmon scattering models also predict polarisation directions related to the source geometry.

(7) Can we identify the time when a given outburst was initiated? Brightness temperature limitations generally preclude the possibility of seeing the start of an outburst at radio wavelengths. However, an unusual change in the optical or X-ray output may serve to identify the time origin (for example, in 3C273) which is particularly important in the case of screen models as this would allow a unique inversion of the geometry of the screen if the signal velocity is taken to be c .

(8) Is the position angle of compact double structure aligned with extended radio structure? So far the evidence for this is positive and so this constitutes an argument in favour of the extended sources' being fuelled continuously⁵⁴ by their central components. In these cases it is tempting to suppose that both sources share a common axis defined by the angular momentum of the central regions. However, as yet amongst these sources, only in 3C273 is there evidence for super-luminal expansion and it is very important to discover more examples. This is because, in most extended double radio sources, one can be fairly sure that the axis does not make a small angle with line of sight. So if rapid expansion, with $v_0 \gg c$ are found to be fairly common, it would probably rule out both ballistic and simple

screen models. One important source to study in this context is Cygnus A because in this case there is fairly convincing evidence that the source axis lies in the plane of the sky⁵⁵.

(9) What are the statistical properties of a sample of compact sources? It is already known that $\geq 10\%$ of flat spectrum 3C sources display super-luminal expansion. In some models where ejections occur in a particular direction and Doppler effects are important, the compact components would appear undetectably faint unless our line of sight lies in a privileged range of solid angle. As we have discussed, the probability of this may be related to the observed expansion speed in which case it should be possible to rule out this type of model. (Note, however, that there is a possible selection effect in that sources exhibiting large Doppler shifts will be preferentially included in a catalogue such as 3C.) We doubt that any models of the screen or ejection type could accommodate regular expansions with speeds $v_0 \gg 10c$ in more than a very small fraction of a large sample of sources. If these velocities were observed then they should motivate attempts to develop less unsatisfactory forms of models in alternative categories. On a more ambitious level, if sufficient understanding of the kinematics of a class of sources can be achieved, it may be possible to invert the problem and set limits on plausible values of the Hubble constant (or conceivably q_0).

Finally, we would stress the importance of carrying out systematic multi-frequency source monitoring using a standard (although necessarily somewhat arbitrary) algorithm for converting raw visibility data to source models, and of pursuing programmes that use the same combination of telescopes for successive observations.

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articles

Structure of RNA and RNA binding site in tobacco mosaic virus from 4-Å map calculated from X-ray fibre diagrams

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The structure of tobacco mosaic virus has been solved to a resolution of 4 Å using fibre diffraction methods. The general fold of the protein is clear, the conformation may be deduced in many places, and the conformation of the RNA and its interaction with the protein can be seen.

Tobacco mosaic virus (TMV) is a rod-like virus, 3,000 Å long and 90 Å in radius, with a central hole of radius 20 Å. The identical protein subunits (molecular weight 17,500) build

a helix of 49 subunits in 3 turns, protecting the RNA. The particles form very well oriented gels^{1,2} which give X-ray fibre diffraction patterns of unsurpassed quality^{2,3}. The first successful calculation of a three-dimensional electron density map was by Barrett *et al.*⁴; however, they limited their analysis to the cylindrically symmetric part of the diffraction pattern, with a resolution of 10–15 Å. They pointed out that with sufficient heavy atom derivatives, the diffraction pattern could in principle be analysed at higher resolution, and Stubbs and Diamond⁵ showed that this could indeed be done. Holmes *et al.*³ applied these methods, to produce a map at 6.7 Å resolution. We report here an extension of the 6.7-Å work: data have been collected to 4 Å resolution and the heavy atom positions have been further refined, producing significant improvements in

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Table 1 Heavy atom derivatives

Ligand	Strain	Site no.	Residue	<i>f</i>	<i>r</i> (Å)	φ (deg)	<i>Z</i> (Å)	<i>R</i> (%)
OsO ₄	Vulgate	1	1	80	92.0	5.5	8.5	12.7
Pb ²⁺	Vulgate	1		50	26.0	6.5	20.2	12.3
UO ₂ F ₆	Vulgate	1	27	70	57.2	15.5	12.6	15.0
		2		50	26.8	5.9	19.1	
		3		55	57.5	22.3	25.4	
SHIMS-MMN	Vulgate	1	68	60	72.4	5.7	— 1.5	16.6
		2	27	70	56.7	15.5	12.6	
MMN	Vulgate	1	27	70	56.9	15.5	12.6	15.0
MMN	Ni-2068	1	27	70	57.1	15.5	12.6	13.8
		2	139	70	72.6	0.4	0.5	

SHIMS, sulphhydryl imidoester²²; MMN, methylmercury nitrate⁸. Units of *f* are arbitrary, and correspond approximately to electrons. *R* is a least-squares *R* factor²³. *r*, φ and *Z* are cylindrical polar coordinates.

the electron density map. The quality of the inner part of the map seems to be what would be expected from a good 4 Å map and this has encouraged us to fit molecular models for the protein and the RNA to the map. We are considerably helped by the fact that most of the RNA binding site is composed of α-helix. We find little ambiguity in the way the atomic models can be fitted to the electron density map. For technical reasons the resolution of the map falls beyond about 60 Å radius so that we have not attempted to build detailed models for this region, although the trace of the polypeptide chain may be seen for the whole of its length.

The application of heavy atom isomorphous replacement to the fibre diagram of TMV has been described^{3,6}. The essential steps are data collection^{3,6}, location of heavy atom sites, calculation of phases and separation of Bessel functions, and Fourier-Bessel inversion⁹. These last two steps are equivalent to reconstructing three-dimensional diffraction data from the observed two-dimensional data. The nature of cylindrically averaged data precludes the use of Patterson methods in locating heavy atoms. Consequently, heavy atom location has been one of the major obstacles to progress in this work. The methods finally used depended on determining the occupancy and radius of a heavy atom from the zero layer line⁷, then finding the other coordinates from the remaining layer lines^{3,8}. Single site derivatives were essential in the early stages, as were double derivatives of these. The heavy atom derivatives used are described in Table 1.

The phase problem for cylindrically averaged data differs from that in crystallography. The intensity of diffraction is⁹⁻¹¹

$$I = \sum_n \mathbf{G}_n \mathbf{G}_n^* \quad (1)$$

G is a vector analogous to the crystallographic *F*. The allowed values of *n* depend upon the helical symmetry of the particle. Three terms of equation (1) should be considered for TMV at 4 Å resolution. Thus the intensity contains six unknown quantities, the real and imaginary parts of three *G_n*, c.f. whereas in crystallography there are only two unknowns. The solution of this multi-dimensional phase problem has been described by Stubbs and Diamond⁸ and Holmes *et al.*³

Electron density map

The map shows clearly recognisable right-handed α helices and resolved sugar and phosphate peaks in the RNA. We have examined 4-Å maps of proteins and a 4-Å map of tRNA produced by Rich and his colleagues, and conclude that by current standards, this is a good protein map, and a particularly good RNA map for this resolution. The exception is the region at radius greater than 65 Å, where the resolution is reduced in the azimuthal direction, falling to 5.5 Å at 80 Å radius (G. S. & S. W., unpublished). However, parallel studies on the crystallised disks of the coat protein¹² will yield detailed information about the outer region, so we have not attempted a detailed fit beyond 60 Å radius.

The RNA stands out as the strongest and clearest feature.

It follows the virus helix at a radius of about 40 Å, as has long been known²⁴, but it is considerably puckered, and in fact lies between 35 and 50 Å. It is a continuous chain, but with a number of interactions with the protein density, and three protruding features which we identify as bases. There are clearly separated peaks along the chain, interpreted as sugar rings and phosphates. The RNA density (without two of the protrusions, which are obscured by the main chain in this view) is shown in Fig. 1a.

The protein chain (158 amino acids) can be traced with confidence for about 70% of its length, from residue 20 to 55, and from 69 to 140. The connecting peptide, the N-terminal chain, and the C-terminal chain are present, but details are less certain, since these all lie at high radius. There are four α helices running approximately radially, which have been described at lower resolution³ and have also been seen at lower resolution in the crystallised coat protein¹². We adopt the descriptive terminology of Champness *et al.*¹² and call these the left and right radial helices (LR and RR), and the left and right slewed helices (LS and RS). Part of the electron density corresponding to the left radial helix is shown in Fig. 1b. It is clearly a right-handed α helix (confirming our choice of hand, made on the basis of the electron microscopy results of Finch¹³) and most of the side chains are marked by density, although not, of course, sufficiently well to recognise them. The same is true of the left and right slewed helices. The right radial helix is not as clear as the other three but is recognisable by its long, straight form, its size and density, and a certain amount of periodicity.

There is also a short stretch of helix at 23 Å radius, the innermost boundary of the protein. We call this the vertical helix (V). This runs parallel with the particle axis, and packs closely with its equivalent helices in adjoining subunits to line the central hole of the virus. Figure 1c shows the density at low radius, where three helices including this one form a bend.

Although the connectivity of these helices was not correctly ascertained at 6.7 Å resolution in the virus³, it was clear at 5 Å in the protein crystals¹² and the chain tracing of Champness *et al.*¹² is confirmed here at 4 Å. In addition, we can trace the chain at low radius, a region which lacks density in the protein map, probably because of disorder¹². The LS helix, beginning near Pro 20, runs inward. It may be slightly shorter than its counterpart in the absence of RNA (see Fig. 1 of Champness *et al.*¹²). It connects to the second helix (RS), which runs from about 50 Å radius outwards, then the chain goes through RR to very low radius, almost meeting the vertical helix, after which the LR helix leads to high radius at about residue 135. The remaining residues are at high radius.

Model building

Protein model building at 4-Å resolution is not normally possible. In the case of TMV, however, many constraints are provided by the abundance of chemical information. Moreover, the map is very clear. The locations of residues Ser 1, Cys 27, Lys 68, and Tyr 139 were fixed by the binding of specific heavy

atom derivatives at those sites^{4,7}. The C terminus is known to be on the outside surface because it is accessible to carboxypeptidase¹⁴. Especially useful were the sequences of over 100 mutants and strains of TMV, indicating which residues are invariant. It was helpful to know the 5 Å structure of the crystalline protein disk¹², which clearly shows the path of the

polypeptide chain connecting the LS and RS helices (residues 31–41), a region which is ambiguous in our map. A most important factor in our ability to build a model was the fact that at radius < 60 Å, TMV is mostly α helical. The atomic model was built with Nicholson push-fit models (1 cm to 1 Å) in a Richards box¹⁵.

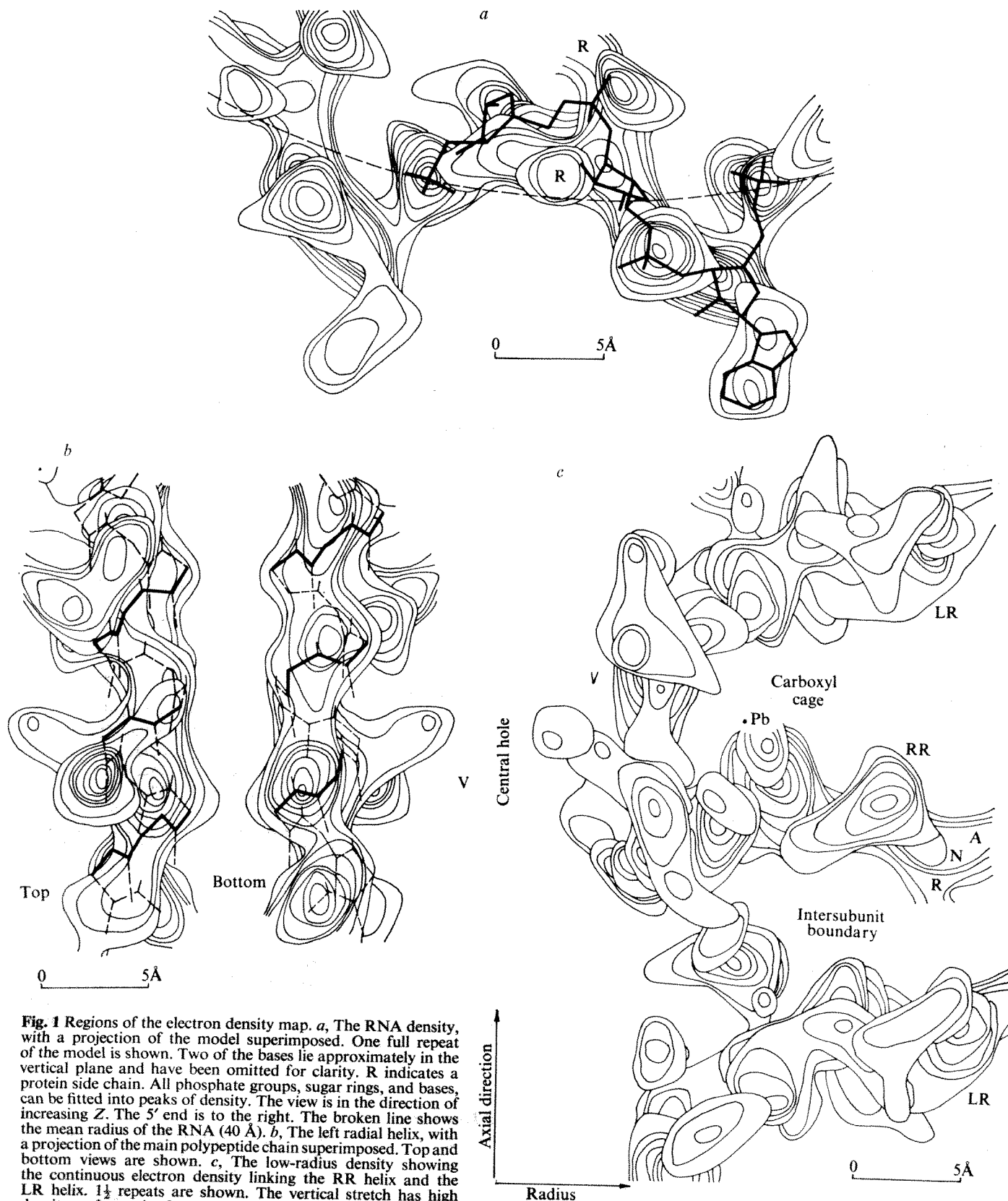


Fig. 1 Regions of the electron density map. *a*, The RNA density, with a projection of the model superimposed. One full repeat of the model is shown. Two of the bases lie approximately in the vertical plane and have been omitted for clarity. R indicates a protein side chain. All phosphate groups, sugar rings, and bases, can be fitted into peaks of density. The view is in the direction of increasing *Z*. The 5' end is to the right. The broken line shows the mean radius of the RNA (40 Å). *b*, The left radial helix, with a projection of the main polypeptide chain superimposed. Top and bottom views are shown. *c*, The low-radius density showing the continuous electron density linking the RR helix and the LR helix. $1\frac{1}{2}$ repeats are shown. The vertical stretch has high density and may be fitted to a 10-residue α helix (V) which is terminated by Pro 102 at the N-terminal (RR) end.

Table 2 RNA conformation—angles used for model building

Nucleotide	ϕ (deg)	ψ (deg)	θ (deg)	ξ (deg)	σ (deg)	ω (deg)	χ (deg)
1	70(g^+)	145(t)	220(t)	170(t)	135(C2'-endo)	250(g^-)	110($anti$)
2	275(g^-)	40(g^+)	185(t)	195(t)	135(C2'-endo)	210(t)	170($anti$)
3	290(g^-)	150(t)	135(t)	270(g^-)	135(C2'-endo)	210(t)	105($anti$)

The angle values are unrefined and thus somewhat uncertain. For definitions of angles see Arnott (ref. 16).

The RNA

The RNA has three nucleotides per protein subunit. In the map there is a separate local maximum for every phosphate, every ribose and every base. In order to be certain that only one RNA structure would fit the map, we systematically tried and rejected all but one of the permutations of previously observed conformations of the variable angles. Following the notation of Arnott¹⁶, we used the constraints (1) (ϕ , ψ) $\approx$ (180°, 180°) is not allowed^{17, 18}. (2) $\xi \approx 300^\circ$ implies $\sigma \approx 140^\circ$ (C2'-endo)¹⁹. (3) All bases are *anti*^{16, 18}. (4) All other angles are within or near the previously observed ranges for these angles, as compiled by Arnott¹⁶. (5) Only C2'-endo and C3'-endo conformations were tried as the pucker of the ribose ring; the other possible puckers were judged to differ so slightly from one or the other of these as to be indistinguishable by our methods. (6) Both possible directions of the RNA chain were pursued.

Each conformation tried was set first at a nominal angle and then small variations of the angles were used to improve the fit if possible. Of the approximately 130,000 possible permutations, only about 1,000 had to be tried because many trials were rejected after setting the first few bonds. We were only able to find one conformation which fitted the density. As a check we note that this conformation has the property of being able to repeat as a continuous chain into the next subunit, as required. The geometry of the RNA is given in Table 2. A projection of the RNA, superimposed on its electron density, is shown in Fig. 1a. All three sugars were found to have the C2'-endo conformation. This conformation is found in B-form double helical DNA¹⁶ (ref. 16) and in non-helical regions of tRNA (ref. 18). The three phosphates are at radii 37, 39.5, and 42 Å. The 5' end is at the concave end of the particle. This result has been independently obtained from electron microscopy by Wilson *et al.*²⁰.

The protein

Each helix was first fitted as a polyalanine helix to establish the course of the backbone and to fit β -carbons into bumps of density.

Left slewed helix, residues 21–31—Pro 20 was placed at the bend where the helix starts. The phase of the helix was specified by requiring Cys 27 to be near the MMN site.

Right slewed helix, residues 40–53—Pro 54 was placed at the bend ending this helix. The phase of the helix was chosen to place Phe 48 and Trp 52 into density peaks corresponding to their shapes. This placed Arg 41 near phosphate 1, Glu 50 on the intersubunit boundary, and a hydrophobic face toward Cys 27.

Left radial helix, residues 114–134—The coordinates of residue 139 were known from a specific heavy atom derivative at that site⁴. This site is 22 Å from the corner which ends the LR helix, so the corner residue is likely to be in the range 126–132. A side chain bump of density near that corner, one turn in from the high radius end of the helix, seems to be a ligand for the third UO_2F_6 site. Residues 128–134 are all hydrophobic except for Glu 131 and Arg 134. Since carboxylic acids are known to bind UO_2^{2+} , we placed Glu 131 at that site, which then specified the phase of the LR helix. This positioning required the external face of the helix to be surprisingly hydrophobic. We see below, however, that it functions as a hydrophobic binding site for the RNA bases.

Vertical helix, residues 103–112—It was already known from the radial density distribution of TMV reconstituted protein^{21, 24}

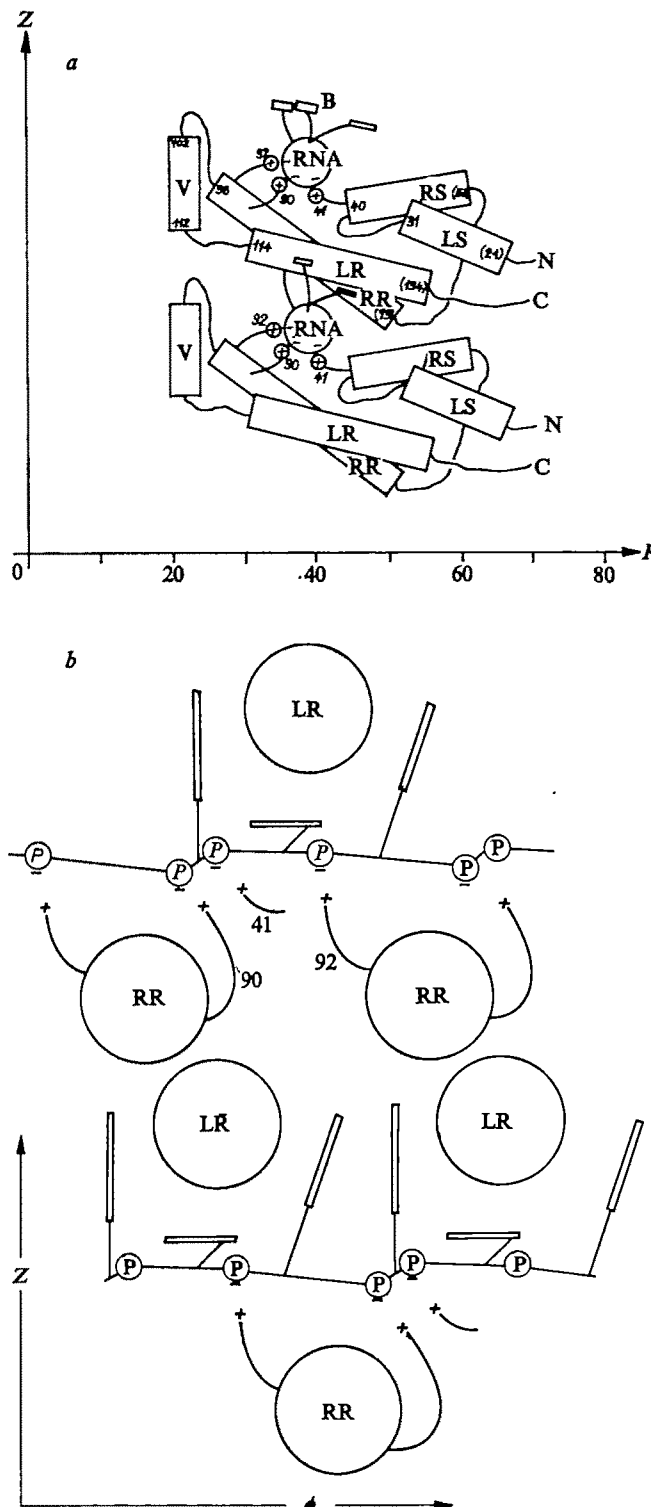


Fig. 2 a, Schematic drawing of the inner part of TMV. The carboxyl cage is formed by the left radial, the right radial, and the vertical helices at a radius of about 30 Å. The view is in the direction of increasing ϕ . B indicates an RNA base. b, The RNA binding site viewed along a radius. The residue numbers of arginines which seem to be forming salt bridges to the phosphates are given. Note how the three bases form a claw which grips the hydrophobic LR helix: LR, left radial helix; RR, right radial helix; P, phosphate.

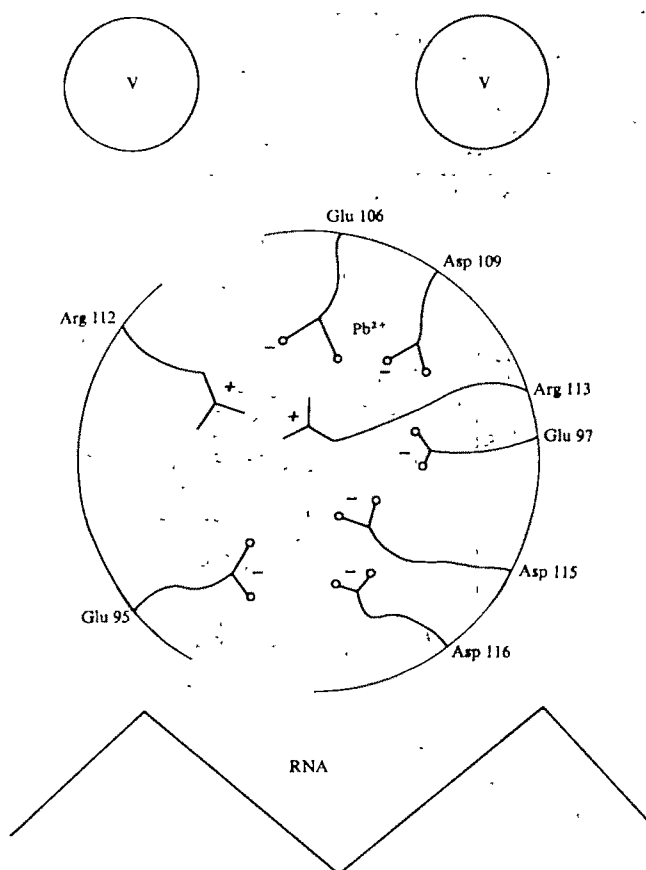


Fig. 3 Schematic drawing of the carboxyl cage as seen from above.

that the region at 23 Å radius was the densest part of TMV protein. Here we see density which we attribute to a 10-residue α -helix parallel to the cylinder axis. At this radius the 22° wedge-shaped subunit is only 9 Å wide, just the proper spacing for close packing of the vertical helices from adjacent subunits. The important constraint on this helix was that Pro 102 should not be built into it. To connect V with LR (and later to pack residues in density between V and RR), we put Pro 102 at the beginning of the V helix. This choice is supported by the fact that this helix is absent or greatly reduced in size in the U2 strain of TMV²² (and S. Morris, G. S. & S. W., unpublished) which has a proline as residue 107. The phase of the helix was specified by fitting the proper side chains best into density. This fit resulted in the Pb binding site being located between carboxyls Glu 106 and Asp 109 and placed four threonines (103, 104, 107, 111) on the surface exposed to solvent of the central hole (radius 19 Å). Since this assignment of phase has important implications for the existence of a 'carboxyl cage' (see below) experiments with specifically labelled mutants are being undertaken to check this point.

Right radial helix, residues 79–96—The requirement that RR connect with V approximately specified the end residue of RR. The respective consequences of building RR to terminate at each residue in the range 92–101 were noted. The phase which provided the best knobs-into-holes packing with LR put the end of RR at 96. This phase also placed the charged head groups of Arg 90 and Arg 92 into density peaks near phosphates 2 and 3 respectively. At Pro 78, at the high radius end of the RR helix, the chain seems to make a slight bend (becoming steeper in Z) and then to continue out in radius as far as residue 70.

Connecting links—Plausible connecting links were built from LS to RS, from RR to V and from V to LR. For residues 98–101, (ϕ , ψ) pairs were set to be in any of the three major allowed regions of the Ramachandran plot²³ and all 81 permutations were tried. Only one was found which both fit the

density and spanned the required distance. The connection between helices LS and RS (residues 32–40) contains many polar side chains which probably form part of the intersubunit boundary toward the left.

Principal features of the structure

From the model it is evident that the part of the molecule we have built includes four significant regions: the packed helices at medium radius, the RNA binding site, a 'carboxyl cage' and the vertical helix. They are shown in Fig. 2a. We discuss them in order.

The packed helices—As has already been observed¹⁸, the four approximately radial helices pack well together, making angles of 10–20° with each other. Our model has good knobs-into-holes interactions. In the vicinity of Cys 27, the helices form the walls of a hydrophobic pocket: the sulphhydryl is in the centre. The unusual unreactivity of the sulphhydryl has long been known²⁴, and is evidently caused by this environment. This cysteine is conserved in most strains and mutants although its function is not clear. In the cow-pea strain, however, it is replaced by a leucine,²⁵ and since this is of similar size, we might guess that the constraint on residue 27 is the packing of the helices.

The RNA binding site—The RNA lies between subunits in successive turns of the virus helix, so that the binding site is in two halves, between the top of one subunit and the bottom of the next (Fig. 2). One half is the base binding site, located almost entirely on the LR helix. This helix has a large number of hydrophobic aliphatic residues between 117 and 128, which form three faces for the bases. All can accommodate any type of base, since the bases lie flat against hydrophobic side chains. Several residues near the faces, including Thr 89, might form hydrogen bonds with the bases, but the model is not sufficiently accurate to make any definite statement. The other half of the site is mostly on the RR helix, and its principal function is to bind phosphate groups by forming ion pairs, using arginines 90, 92 and 41. The hydrophobic chain of Arg 41 might well be stabilised by interaction with Phe 87, which could occupy a peak of density between the RS helix and the RNA. We note that two other groups on the RR helix, Asn 91 and Asn 98, are in positions where they could form hydrogen bonds with phosphate oxygens, but at this resolution we cannot prove that they do so.

The carboxyl cage—Between the RNA and the vertical helix is the feature illustrated in Fig. 3. Six carboxyl groups are in a pocket which is otherwise lined with hydrophobic residues. The pocket is formed by the LR, RR and V helices of one subunit, and the RR helix of the neighbouring subunit. They are partly neutralised by Arg 112 and Arg 113 (coming from different subunits). It seems likely that lead and uranyl fluoride bind to two or more of these carboxyl groups, and that the carboxyls could form a binding site for other divalent cations. Such binding sites have been observed in other structures^{26–27}, but this is a somewhat unusual example, because of the number of carboxyls involved and because the carboxyls are apparently not fully neutralised by bound metal ions or basic side chains. (Nearby, but apparently not interacting with the carboxyl cage, are Asp 88 and Arg 122, which form an inter-subunit salt bridge.) Caspar²⁸ suggested the presence of pairs of carboxyl groups in TMV with anomalous pK values near 7, and this work was confirmed by Butler, Durham and Klug²⁹. There seems little doubt that the anomalous pK values originate in the carboxyl cage, where carboxyl groups are forced together in a hydrophobic environment, although the situation is apparently even more complex than simple pairs of carboxylic acids. The short vertical helices form a wall, part of the rigid carboxyl cage structure, and protect the RNA from the solution.

TMV assembly

From the above observations, we draw some inferences about the assembly and dissociation of TMV. Caspar²⁸ has suggested

that the carboxyls with anomalous pK values provide a sensitive switch, active in physiological conditions, between the dissociated and intact virus states. Considering the RNA binding region and the carboxyl cage together, we can speculate about the mechanism of this switch. The coat protein has two important states, helix-forming and disk-forming, which are delicately balanced in normal conditions. Switching between the states is controlled by pH , presence or absence of RNA, and possibly by concentration of one or more cations.

The mechanism of the switch seems to involve electrostatic interactions among six carboxyls, five arginines, and when present, three RNA-phosphate groups. The helical state exists either in the intact virus or at low pH . It is characterised by the presence of the interlocking V-helices which force carboxyls together in the 'carboxyl cage', accounting for the abnormal proton binding. In the virus, RNA interactions stabilise this state over a wide range of conditions, even above pH 7 where ionisation of some of the abnormal carboxyl groups can be induced. The disk may be obtained at neutral or high pH in the absence of RNA. Comparing our results with those of Champness *et al.*¹³, the disk is characterised by having no V helix and only half the RR helix. Because of the collapse of the RR helix Arg 90 and 92 are presumably able to neutralise carboxyl groups. This rearrangement (involving about 30 residues) is seen as disordering at small radii in the disk-crystals. Through this mechanism the binding energy of the RNA is partially balanced against the repulsive energy of the 'abnormal carboxyls' which aids uncoating. In the low pH helical-aggregate of the coat problem, where protons establish electroneutrality in the carboxyl cage, the protein can adopt the helix structure, even in the absence of RNA (21, 34, and a 4-Å difference Fourier—E. Mandelkow, S.W. & G.S., in preparation). This suggests the formation of the V-helix may be the factor controlling the disk-helix transition.

Finally, in view of the solid wall formed by the interlocking V-helices, the disordering of the V-helix in the disk, which is an important precursor in the assembling of the virus, is probably necessary to enable the RNA to enter its binding site from the inside hole of the growing virus^{30,31}.

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Note added in proof: Further examination of our model shows that Arg 113 (from the subunit at higher Z) could interact with a phosphate of the RNA. This would take Arg 113 out of the carboxyl cage, and take Arg 41 away from the RNA. Arg 90 would bind to the high-radius phosphate, and Arg 113 to the low-radius phosphate. The structure would be otherwise unaltered. At present we are unable to distinguish between these alternatives.

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Transmembrane channel formation in rhodopsin-containing bilayer membranes

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Rhodopsin has been incorporated into planar lipid bilayer membranes. The effect of light is to increase the bilayer permeability in a pattern consistent with the formation of a transmembrane channel of about 10 Å diameter. A model of visual excitation based on a light-activated and voltage-sensitive channel is presented.

PHOTORECEPTOR cells in both vertebrate and invertebrate species are capable of converting a single absorbed light quantum into a neural signal^{1–3}. The photon is absorbed by rhodopsin, a chromophore-bearing membrane protein⁴. The central problem, therefore, is to determine how a

detected photon can initiate an amplified excitatory response of the receptor cell. It is generally accepted that the light-induced changes in rhodopsin lead to cellular excitation by modulating the ionic conductance of photoreceptor cell plasma membranes^{2,3,5–7}. Such changes could result from a simple mechanism based on rhodopsin functioning as a light-activated ion translocator⁸. A direct way to test this possibility would be to incorporate rhodopsin into a membrane system amenable to the experimental methods required to investigate the postulated transport properties^{9,10}.

Here we describe the properties of planar lipid bilayer membranes with rhodopsin included, and show that light increases the membrane conductance in a pattern consistent with the formation of a transmembrane channel^{10–14}. We propose a heuristic model of visual phototransduction which accommodates the data so far obtained in photoreceptor cells.

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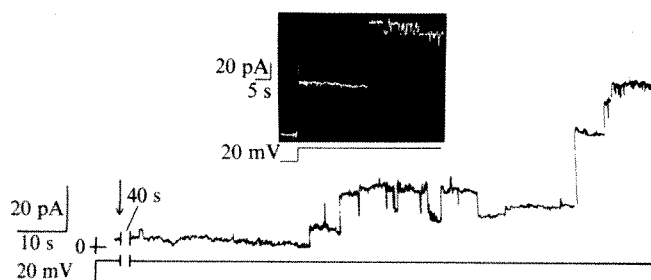


Fig. 1 Time course of the current transition from dark to bleached states. Planar bilayers were formed in a circular aperture of 0.26 mm in diameter as described before²⁹. The Teflon cell consisted of two compartments, each with an area of 3.5 cm². Calomel or Ag-AgCl electrodes were connected to a feedback current amplifier of the type previously described³⁰. The cell and the current amplifier were enclosed inside a light-tight aluminium box. A polarising d.c. voltage, of various amplitudes, was applied across the membrane, the current was displayed on a storage oscilloscope and simultaneously recorded on a strip chart recorder. Bleaching was accomplished with 500 nm light of approximately 10^{16} quanta cm⁻² s⁻¹. All bilayer experiments were done at room temperature. Rod outer segments from dark-adapted bovine retinas (Hormel) were isolated by sucrose flotation and purified in a discontinuous sucrose gradient³¹. Cetyltrimethylammonium bromide (CTAB) was used to extract rhodopsin³². All the steps were performed at 4 °C under dim red light unless otherwise stated. The rhodopsin proteolipid was prepared as follows: detergent (CTAB) solubilised rhodopsin (0.75 mg) was incubated with humid Bio Beads SM-2 (ref. 33) (90 mg) for 5–7 min at 24 °C, and then mixed with a dispersion of partially purified soybean phospholipids³⁴ 10 mg ml⁻¹ in 1.0 ml of 0.1 M KCl, 0.01 M imidazole buffer, pH 7.0. The mixture was sonicated by immersion of the test tube in a water bath sonicator³⁵ for 4 min at 4 °C. Then 0.1 ml of 100 mM CaCl₂ and 1 ml of hexane were added to the suspension. The tube was vigorously mixed for 4 min and the two phases were separated in a clinical centrifuge for 1 min. The hexane phase was removed and a second extraction was performed with 1 ml of ethylether; the suspension was mixed for 3 min and the two phases were separated. The two extractions were performed at 4 °C. The hexane extract exhibits a mean absorbance difference, ΔA_{500} (dark-bleached) of 0.022 ± 0.021 ($n = 20$), whereas the ether extract presents a mean $\Delta A_{500} = 0.183 \pm 0.043$ ($n = 20$). An ether rhodopsin proteolipid with $\Delta A_{500} = 0.16$ was spread over 0.2 M KCl, 1 mM CaCl₂, 0.01 M imidazole pH 7.0. After evaporation of the ether the bilayer was formed. In the figure the membrane current at a voltage of 20 mV is displayed. The arrow represents the point at which the light was switched on.

Planar bilayers which separate two well defined aqueous phases offer the possibility of directly measuring electrical parameters: the time course of light-induced conductance changes together with direct evidence for channel existence can provide insight into the mechanism of rhodopsin action.

Rhodopsin-containing bilayers were made from the rhodopsin proteolipid in organic solvent as described in the legend of Fig. 1. Successful regeneration of rhodopsin treated by this procedure (yield ~70%) showed that it displayed normal spectral characteristics of the native form. The principle of hydrophobic apposition of two monolayers was used for bilayer formation^{9,10,13}. The absolute membrane conductance (G_m) in the dark was in the range $0.2\text{--}2 \times 10^{-9} \Omega^{-1}$; on illumination it increased to a variable extent. Figure 1 is a representative strip-chart recording of the kinetics of conductance transition from the level in the dark ($G_m = 2.1 \times 10^{-10} \Omega^{-1}$) to that in the light. The lag time between illumination and onset of the conductance transition is about 100 s. Estimated half-time of bleaching with the 500 nm light used here is about 3 s. Note that the conductance change proceeds in steps of magnitude of about $4\text{--}8 \times 10^{-10} \Omega^{-1}$ and also in bursts of large magnitude. The difference between the conductance steps and the bursts is more clearly illustrated in the inset, where an oscillographic recording of the same membrane is taken 330 s after illumination: the initial G_m is $32 \times 10^{-10} \Omega^{-1}$, the burst of con-

ductance of $40 \times 10^{-10} \Omega^{-1}$ and the fluctuations in the upper level of G_m are of $5.8 \times 10^{-10} \Omega^{-1}$.

The G_m transition from dark to bleached level may proceed in a 'noisy' way where no identifiable fluctuations can be resolved; this pattern was prevalent in the bilayers prepared from proteolipids with the highest rhodopsin concentration. G_m transition frequently proceeds in steps and bursts concurrent with a drift towards high G_m values. When the dark G_m was initially considerable, or in bilayers formed from a rhodopsin proteolipid which had been purposely bleached, the membrane conductance showed clearly defined fluctuations indistinguishable from those displayed in bleached membranes after the dark to light transition (see below).

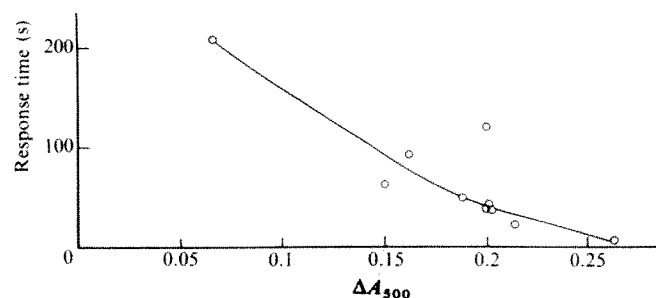
This conductance pattern can be recorded in symmetrical media containing either KCl or NaCl as the electrolyte. Ca²⁺ enhances the conductance changes recorded in planar bilayers¹⁰: in the presence of Ca²⁺, the frequency of the conductance fluctuations and the highest levels of conductance reached upon illumination are higher than in its absence.

A distinct latency period between illumination and onset of conductance changes was characteristic in all experiments. Figure 2 illustrates the dependence of the bilayer response time on the rhodopsin concentration present in the proteolipid. Although the total amount of rhodopsin in the proteolipid is known, we have no way of determining the rhodopsin concentration in the bilayer. The latency period varied from 3–200 s and was shorter the higher the rhodopsin content in the proteolipid extract.

In experimental conditions of a steady-state voltage of 20 mV, and a 0.2 M NaCl, 1 mM CaCl₂ solution, three main types of transitions appeared, with amplitudes of about 2, 4 and $6 \times 10^{-10} \Omega^{-1}$ (Fig. 3). The distribution of G_m transitions was equivalent in dark and bleached membranes (Fig. 3). Thus, illumination does not change the fluctuation pattern, it merely increases the absolute conductance and the frequency of fluctuations. A current trace showing the three transitions is shown in Fig. 4. These results indicate the light-induced formation of a channel by rhodopsin.

The individual conductance transitions were ohmic up to 100 mV (Fig. 5). In contrast, the macroscopic G_m was not ohmic but voltage dependent. This is illustrated in Fig. 6: at voltage steps of ≤ 20 mV, G_m displays a steady value with fluctuations of the established magnitude; at voltages greater than about 20 mV the initial G_m relaxes to lower steady-state values. The curve in Fig. 6 shows a region of low voltage sensitivity between 0 and 20 mV. At higher voltages a steep drop of the normalised conductance occurs reaching almost zero. This behaviour establishes the voltage sensitivity of the rhodopsin channel, that is, at high voltage, the channel closes.

Fig. 2 Dependence of the bilayer response time on the rhodopsin content of the proteolipid. Bilayers were prepared from ether proteolipids exhibiting different ΔA_{500} . The time after bleaching required to detect a definite conductance increment is presented in the ordinates. Other experimental conditions as Fig. 1.



As seen from the insets of Fig. 6 the time constants τ_c of closing are in the order of seconds: for example, at a clamping potential of 50 mV the main part of the closing process occurs with a $\tau_c \approx 3$ s. Fast switching the voltage (time constant ~ 30 ms) to zero or inverting the polarity opens the channel. Therefore, the time constant of channel opening is much faster than that for channel closing.

The situation illustrated so far is characteristic of media containing either NaCl or KCl (and 1 mM CaCl_2). But we also made conductance measurements with two other cations— Ca^{2+} and TEA^+ (tetraethylammonium). With both ions in symmetrical 0.2 M solutions, voltage-dependent G_m and G_m fluctuations were seen.

Concentration potential measurements performed in bilayers separating two compartments with different salt

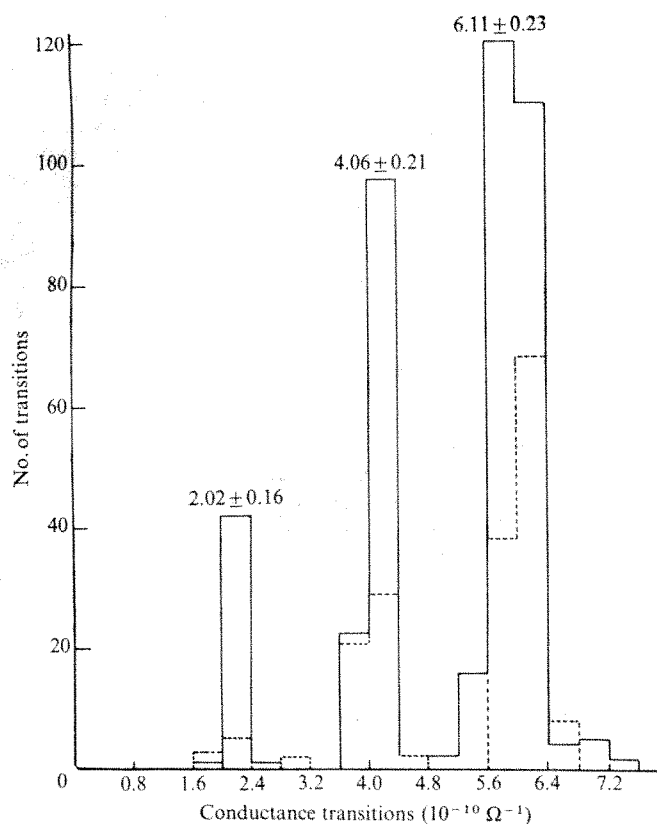


Fig. 3 Histogram of the conductance transitions in dark and bleached states. Only well defined current transitions observed at the steady state voltage of 20 mV in 0.2 M NaCl, 1 mM CaCl_2 , 10 mM imidazole pH 7 of different membranes are collected for the histogram. Two sets of data are presented: transitions in dark (---), and in bleached (—) membranes. Fluctuations of $\leq 0.8 \times 10^{-10} \Omega^{-1}$ and conductance bursts $\geq 8 \times 10^{-10} \Omega^{-1}$ are not included. The ether proteolipids used for the bilayer membranes exhibited ΔA_{500} of $0.17 < \Delta A < 0.26$. Other experimental conditions as for Fig. 1.

activities, showed that G_m is cation selective. Transference numbers for H^+ , K^+ and Ca^{2+} , calculated from the membrane potentials, are 0.85, 0.83 and 0.9 respectively.

The proteolipid in the organic solvent solution contains, in addition to spectrally native rhodopsin, residual detergent, free retinal, and opsin. It is reasonable to ask which of these four components are responsible for the observed effects. Appropriate control experiments were therefore performed: light-induced conductance increases and current fluctuations have been observed with bilayers formed from monolayers of retinal rod disks spread by Trurnit's method, and in this situation, of course, detergent is totally absent^{12,15}. Unfortunately, this system cannot be used

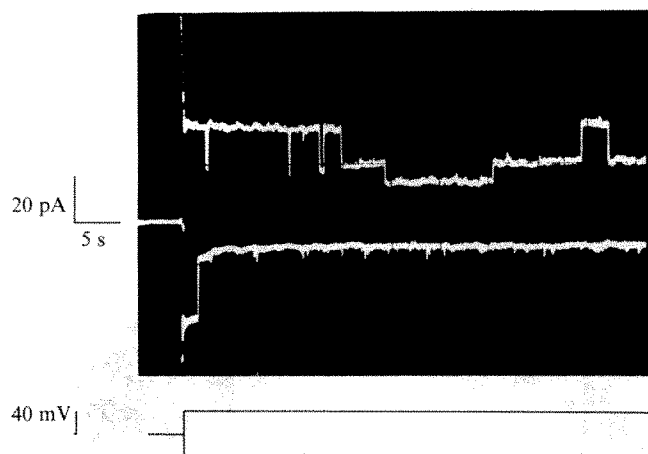
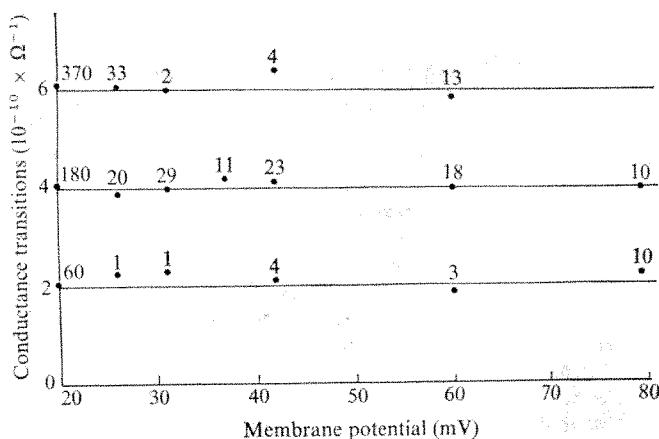


Fig. 4 Membrane current at voltages of +40 and -40 mV from a bleached rhodopsin bilayer. The initial current transient corresponds to the capacitive component. The transition of 2 and $4 \times 10^{-10} \Omega^{-1}$ can be calculated from the positive current record. A transition of $6 \times 10^{-10} \Omega^{-1}$ is recorded in the negative current trace. The G_m fluctuations are not due to steps in bilayer breakdown: they can be recorded even at 10 mV in membranes which are stable up to 3 h. The bilayer was prepared as for Fig. 1.

routinely because of the difficulty in bilayer formation. Alternatively, the recombination of retinal rod disks with phospholipids by sonication allows the extraction of a rhodopsin proteolipid into the solvent with an efficiency comparable with that of the second-ether-extract from CTAB solubilised rhodopsin. It is clear, therefore, that this proteolipid has never been in contact with detergent. Planar bilayers formed from this proteolipid display the same overall behaviour described before in detail: this rules out the participation of the detergent and establishes that rhodopsin is the species responsible of the membrane conductance. The experiments with this proteolipid, which we term 'instant proteolipid', because it speeds up the preparation by obviating the solubilisation step, will be reported in detail elsewhere (in preparation). Another control was done with bilayers formed from proteolipids of opsin (prepared by removal of retinal from bleached rhodopsin¹⁶) which had high conductance ($G_m \sim 10^{-9} \Omega^{-1}$), displayed conductance fluctuations of 2, 4 and $6 \times 10^{-10} \Omega^{-1}$, exhibited a voltage dependent reduction of G_m and, in their overall behaviour, were indistinguishable from those prepared from

Fig. 5 Voltage-dependence of the conductance transitions. The small numbers represent the number of events. Note that the number of transitions decreases as the voltage increases. Experimental conditions as for Fig. 1.



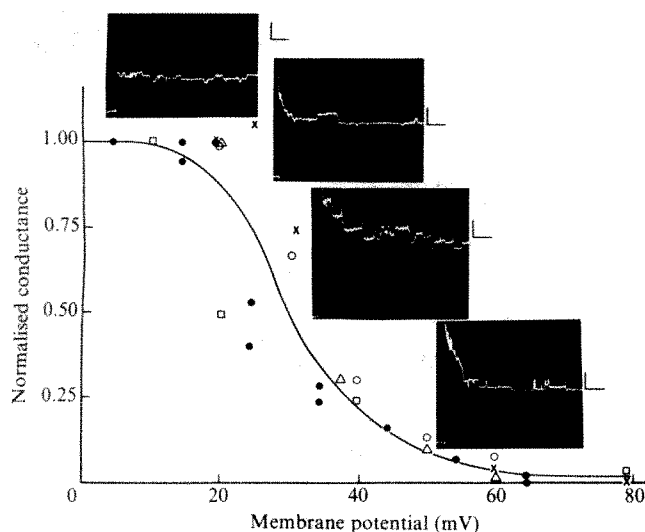


Fig. 6 Voltage dependence of the normalised membrane conductance (G_m at given voltage/ G_m at zero voltage). The figure contains insets of photographs of the current relaxation for voltage steps of 20, 31, 39 and 60 mV, respectively. The lowest line in the photographs represents the baseline at zero current. The initial current peak corresponds to a capacitive transient associated with the onset of the voltage. The vertical scale bars to the right of the photographs represents the current calibration: 50 pA; the horizontal scale bars are the time calibration, 2 s. Two sets of data are presented in the plot: the first set is obtained from four highly conducting membranes ($G_m > 16 \times 10^{-10} \Omega^{-1}$) (●, ○, ×, □); for the second set the four different membranes of low conductance ($G_m < 16 \times 10^{-10} \Omega^{-1}$) (△) were treated as a single membrane.

bleached rhodopsin. As a further control, proteolipids prepared from thermally denatured retinal-free opsin yielded bilayers with low conductance ($G_m < 10^{-10} \Omega^{-1}$); occasional periods of high frequency noise were observed, but these without discernible current step fluctuations. It seems clear, therefore, that the bilayer conductance and its fluctuations are also associated with the presence of opsin. Thus, the variable levels of conductance given by control (that is, dark) rhodopsin-proteolipid bilayers must reflect the presence of variable amounts of bleached rhodopsin (that is, opsin). As noted above, however, whatever the control level of conductance, the effect of light is usually a displacement of the conductance to higher levels.

The planar bilayer experiments have been repeated with proteolipids made from many (>50) different rhodopsin preparations. Light responses of the type shown in Fig. 1 were seen in 30 of the 60 rhodopsin membranes displaying G_m fluctuations. Further technical and analytical refinements should facilitate a more precise characterisation of the bilayer ion-specificity, the kinetics of light-activated responses and light-energy dependence, which could eventually match with the characteristics of photoreceptor membranes².

The light-induced transmembrane channel

It has been shown that light increases the membrane permeability of rhodopsin-containing bilayer membranes. The conductance fluctuations observed in the rhodopsin planar bilayers are of the type induced in lipid bilayers by molecules known to form transmembrane channels^{17,18}. Further, the conductance transitions of the order of $10^{-10} \Omega^{-1}$ indicate a univalent ion flux of 10^8 particles per s for a 100-mV driving force. This calculated flux is higher by four orders of magnitude than the maximal transport rate of an ion-carrier, such as valinomycin, but is the expected value for a channel^{17,18}.

The evidence, taken altogether, establishes that the effect of bleaching rhodopsin in bilayer membranes is to induce the formation of a transmembrane channel. Considering a

channel conductance of $4 \times 10^{-10} \Omega^{-1}$, it is possible to make an order of magnitude estimation of its smallest diameter. Assuming the channel to be a cylindrical pore filled with the bathing electrolyte of bulk conductivity (0.2 M NaCl) and having a length comparable with the membrane thickness of 30 Å, its diameter would be 9 Å (ref. 10). If the channel would be stringently cation selective and using only the Na^+ bulk conductance, its diameter is calculated to be 14 Å. These values seem to be reasonable estimates since the crystal diameter of TEA^+ is about 8 Å (ref. 19) and TEA^+ can pass through the channel. Hydrogen-tritium exchange measurements in rod disk membranes are consistent with the formation of a hydrophilic channel of about 10–15 Å in diameter²⁰, though the data have been recently questioned²¹. Complementary experiments with rhodopsin incorporated into lipid vesicles showed that exposure of the vesicles to light increases the permeability to Na^+ , Cs^+ , Ca^{2+} , glycerol and glucose but not to Cl^- , sucrose and inulin (in preparation). These results indicate that light induces the formation of a wide permeability pathway with a cutoff diameter of about 10 Å. The wavelength dependence of this phenomenon together with a set of control experiments that exclude the involvement of retinal and residual detergent establish that the light response can be accounted for by rhodopsin bleaching¹⁴.

Aggregation can explain the latency of the light response

An immediate obvious difference between the light-induced changes we record *in vitro* and the situation *in vivo* is the relatively long lag period between applications of light and the photoresponse: seconds compared with milliseconds *in vivo*². This difference can be explained by postulating that rhodopsin bleaching changes the permeability by a diffusion limited aggregation process. Such a cooperative process between rhodopsin molecules has been thought to be involved in vertebrate scotopic vision²².

Different models have been considered to explain the experimental observations of the channel and of channel formation in the planar bilayer. A unimolecular channel would be the simplest model. This model suffers from the difficulty of explaining the lag period of the conductance increase after bleaching. The difficulty arises from the assumption that metarhodopsin II, which is produced in milliseconds at room temperature³ (the experimental condition of the planar bilayers), is a conducting species—as seen from the vesicle experiments^{11,14}.

The latency period could be perhaps best explained by a channel formed from rhodopsin units. These aggregating molecules would form after bleaching, and would have to find each other by diffusion in the plane of the membrane. Since the membrane contains only few channels, and thus few aggregating species (assuming a homogenous distribution of rhodopsin), then the observed latency is actually shorter than expected on the basis of unrestricted diffusion. For example, with a diffusion constant of $\sim 10^{-9} \text{ cm}^2 \text{ s}^{-1}$ (ref. 23) for rhodopsin in the bilayer, the molecule travels only 10^{-4} cm in 100 s, that is 1/100 of the actual membrane diameter. To overcome this discrepancy one has to assume that the diffusion occurs in a severely constrained domain. Indeed, examination of the surface layer from which the membranes are formed by phase contrast microscopy, shows patches of the size in question, that is, 10^{-4} cm diameter; they appear white, time stable and probably contain opsin. In contrast, in the disk membrane the protein-protein distance is only tens of Ångströms and so the diffusion-dependent delay is much less than 1 ms, and has no effect on the physiological response time.

Further support for this proposal is that the lag time decreases as the rhodopsin concentration in the proteolipid increases, as seen from Fig. 2.

Once the channel is formed it is fixed and stable. Its

voltage sensitivity cannot be explained by dissociation and subsequent diffusion, since a voltage step to the inverted polarity restores the high conductance state in less than 0.1 s, a time which is considerably shorter than that required for the formation of the channel.

Our favoured model, therefore, is that of a fixed conducting aggregate formed from subunits.

Proposed model of visual excitation

What is the link between the photochemistry of rhodopsin and the bilayer permeability response? The photoisomerisation of the retinal chromophore in rhodopsin triggers transitions in the protein through a sequence of conformational states. The metarhodopsin I to metarhodopsin II transition is the last step in the bleaching sequence which is fast enough to be involved in the transduction process^{2,3}. Since the light-induced permeability in rhodopsin vesicles is elicited at 3 °C, metarhodopsin II may be the first conducting (aggregating) form of rhodopsin.

We have detected capacitative photocurrents in rhodopsin layers from which the bilayers are formed; these arise from charge displacements in rhodopsin as it bleaches²⁴. The photocurrents occur in milliseconds and thus precede the formation of the channel. In analogy to the sodium channel of nerve membranes, where a capacitative—'gating'—current is associated with its opening and closing²⁵, it is tempting to suggest that the measured photocurrents represent the gating current of the rhodopsin channel²⁴.

Irrespective of whether the channel is formed from one or several rhodopsin molecules, it is evident that the amplification gained on allowing the passage of more than 10⁶ ions per s per absorbed photon, is indeed, enormous^{1,2,4,8}.

In vertebrate rods, rhodopsin is located in the intracellular disks and the photoinduced reduction of the 'Na⁺-dark current' occurs in the plasma membrane: a connecting signal between the two membranes is required^{2,3}. Calcium has been postulated as the transmitter²: cytoplasmic Ca²⁺ activity increases during illumination because of a light-induced permeability of the disk membrane to Ca²⁺; Ca²⁺ regulates the 'Na⁺-dark current' by binding reversibly to the plasma membrane sites through which Na⁺ flows into the outer segment. In addition, a Ca²⁺-ATPase has been described in rod outer segments²⁶ and in purified disk membranes²⁷. Based on our findings a model of visual phototransduction can be proposed: in the dark, the channel is closed and the ATPase would pump Ca²⁺ into the disks; on bleaching, the channel

would form and allow Ca²⁺ to diffuse down its concentration gradient. The diffusion of Ca²⁺ would change the disk membrane potential to the value (threshold) at which the channel closes. The channel closure, the photoregeneration of rhodopsin, as well as the activity of the pump, restore the membrane to the conditions existent in the dark. Thus, a light-induced and voltage-sensitive channel constitutes an efficient phototransducer that utilises the well established principles which govern membrane excitability and considers the neural origin of retinal photoreceptors²⁸. There is no evidence at present to extend this scheme to the invertebrate photoreceptors where the situation seems more complex^{2,3}.

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X-ray study of the lithium complex of NAD⁺

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The Li⁺-NAD⁺ complex exists as a 'dimer' of two molecules arranged head-to-tail with Li⁺ coordinated tetrahedrally to adenine N(7) and three pyrophosphate oxygens. Adenine is stacked intermolecularly on nicotinamide. The conformation of NAD⁺ is 'extended' and similar to that found in holoenzyme complexes. This is in contrast to the 'folded' structure proposed from spectroscopic studies.

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BIOLOGICAL redox reactions are catalysed by enzymes requiring cofactors which receive hydride or electrons from one substrate (oxidation) and donate them to another (reduction). Three main groups of enzymes can be distinguished: those containing the (dissociable) coenzymes NAD⁺ or NADP⁺, those with the (non-dissociable) prosthetic groups FAD or FMN, and the cytochromes. The largest group is that of NAD⁺- or NADP⁺-dependent dehydrogenases of which over 150 have so far been studied^{1–3}.

NAD⁺ was first discovered in 1904 by Harden and Young. It was isolated in 1931 by von Euler and co-workers

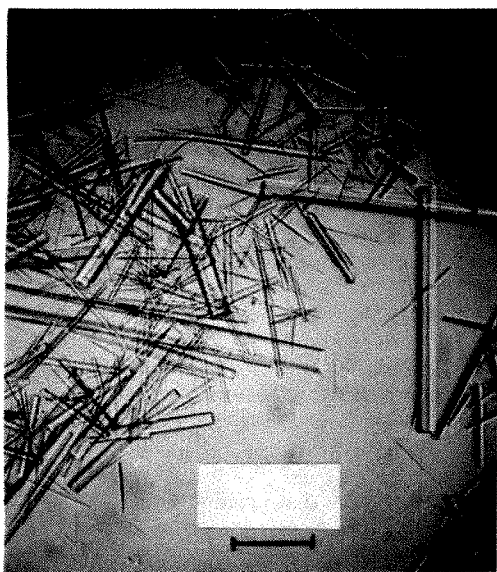


Fig. 1 Crystals of the $\text{Li}^+\cdot\text{NAD}^+\cdot 2\text{H}_2\text{O}$ complex.

and its chemical structure was elucidated in 1936 by Warburg and Christian¹. Since then, numerous spectroscopic studies such as fluorescence^{1,2}, circular dichroism³ and nuclear magnetic resonance (proton, C-13 and P-31)⁷⁻¹⁴ have been performed on NAD^+ , NADP^+ and synthetic analogues^{2,15-17} in order to establish the three-dimensional structure. It was found that in aqueous solution, NAD^+ occurs in a 'folded' conformation with the two heterocycles forming an intramolecular stack. Depending on the stack (adenine on nicotinamide or vice versa), right- and left-handed helices result which are assumed to be in a rapid equilibrium with an intermediate 'extended', non-stacked form (see ref. 10 for review).

More direct information about the structure of NAD^+ was obtained from X-ray crystallographic data of its holo-enzyme complexes with lactate dehydrogenase (LDH), soluble malate dehydrogenase (s-MDH) glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and liver alcohol dehydrogenase (LADH) (ref. 1). All four enzymes bind NAD^+ in a related 'extended' conformation and not in the folded form proposed for NAD^+ in solution. These analyses also revealed that NAD^+ can bind with the nicotinamide residue either in the *syn* or *anti* orientation, depending on the enzyme, and thus hydride transfer to the C(4) position can occur from both sides of the pyridine ring (see ref. 2 for classification of A and B dehydrogenases).

Attempts to crystallise NAD^+ date back to 1957 but

until this present investigation no specimens suitable for an X-ray analysis had been reported¹⁸. In this paper we describe our crystallographic studies carried out on the $\text{Li}^+\cdot\text{NAD}^+$ complex.

Experimental procedure

For crystallisation, 1 g lyophilised NAD^+ free acid was dissolved in 0.75 ml water and adjusted to pH 4.0 by slow addition of 1.3 ml 1 N LiOH. Then 2 ml methanol were added and the solution was kept at 20–25 °C for 30 h. The crystallised product (Fig. 1) was filtered off, washed twice with acetone and dried over P_2O_5 in a vacuum. Yield was 0.9 g (90%) $\text{Li}^+\cdot\text{NAD}^+\cdot 2\text{H}_2\text{O}$, and analysis showed NAD^+ (enzymatic with alcohol dehydrogenase, ADH) at 93.5%; water (by Karl Fischer method), 5.5%; lithium (flame photometric), 1.1%; Fp uncorr., 203–204 °C. $\text{Li}^+\cdot\text{NAD}^+\cdot 2\text{H}_2\text{O}$ is free of inhibitors for the LDH-reaction and the test according to Amador¹⁹ gives 10–15% better results than commercially available ' NAD^+ free acid' preparations.

A crystal of dimensions $0.025 \times 0.025 \times 0.2$ mm was isolated from the methanolic solution, sealed with some mother liquor in a capillary and used for all X-ray studies. Space group and cell dimensions are $\text{P2}_12_12_1$ and $a=10.073(3)$ Å, $b=15.839(4)$ Å, $c=17.821(4)$ Å. Intensity data were collected in the $2\theta/\omega$ scan mode by means of an automated STOE four-circle diffractometer using Ni-filtered $\text{CuK}\alpha$ radiation. Owing to the rapid decrease of intensities with increasing glancing angle θ , only a limited data set corresponding to a resolution of 1.09 Å ($\theta_{\text{max}}=45^\circ$) could be measured. Out of the 1365 data, 338 were below the 3σ threshold and considered as unobserved.

The structure was solved by heavy atom and direct methods employing the multisolution tangent formula approach MULTAN²⁰. Although the most consistent phase set derived with the $450E$'s greater than 1.01 had a rather 'high' Karle R -factor of 50.4%, the resulting E -map showed maxima which could be identified as atoms of the pyrophosphate group and of the adenine heterocycle. The rest of the structure was derived using Fourier techniques. Atomic parameters were refined by full matrix least squares cycles with assignment of anisotropic temperature factors to P and O atoms. The Li^+ ion and some hydrogen atoms could be located from a difference Fourier synthesis at the end of the refinement which finally converged at $R=10.0\%$ for the observed data.

NAD^+ structure

As shown in Fig. 2, NAD^+ is composed of adenylic acid and nicotinamide-5'-ribonucleotide fused together in a head-to-head orientation through a pyrophosphate linkage.

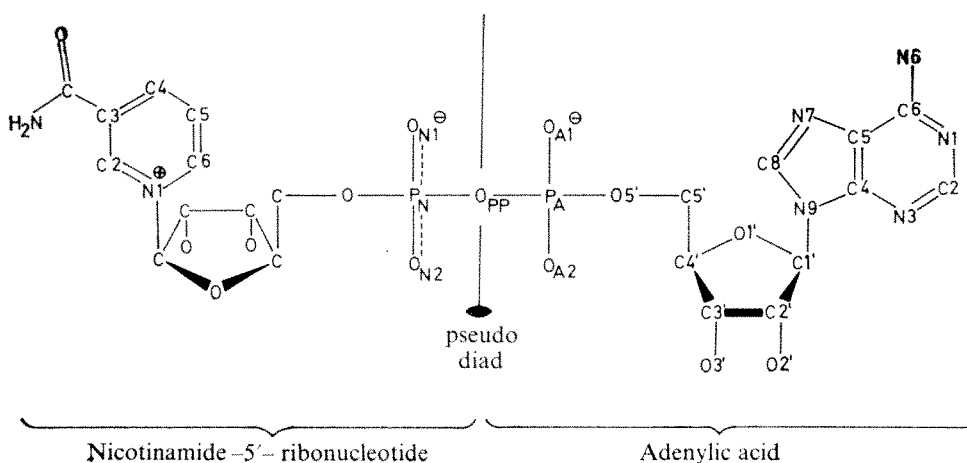


Fig. 2 Chemical structure of NAD^+ and atom numbering convention used. The pseudo diad relating the two ribosephosphate moieties is indicated.

From a structural point of view, NAD^+ could be diad symmetrical (neglecting the difference in heterocycles) with the diad passing through the pyrophosphate O(PP) oxygen atom. In the Li^+NAD^+ complex, however, asymmetry is introduced by two Li^+ cations liganded to the same NAD^+ molecule, one attached to adenine N(7) and a free oxygen atom of the P_N phosphate while the second Li^+ occupies the other free oxygen of this phosphate group and one free oxygen of the P_A phosphate (Fig. 3). In this complex the NAD^+ molecule does not exist in the 'folded' conformation with adenine and nicotinamide heterocycles stacked with each other but rather assumes an 'extended' conformation with the planes passing through the heterocycles in an almost perpendicular arrangement.

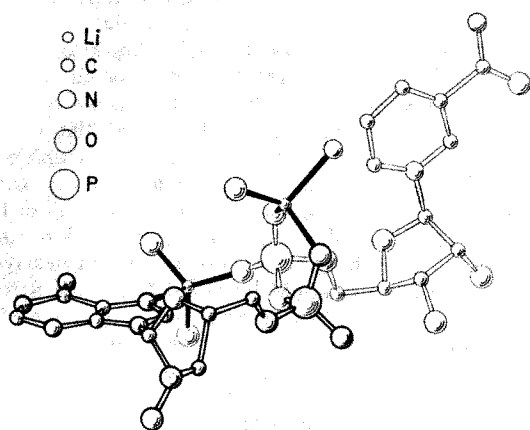


Fig. 3 Molecular structure of the Li^+NAD^+ complex. Coordination to two Li^+ -ions is marked by filled bonds, parts of the molecule nearer to the observer (adenosine moiety) are indicated by heavier lines. Note that adenine and nicotinamide planes are almost perpendicular to each other.

Conformation of the two nucleotides

For 5'-ribonucleotides the energetically most favoured conformational parameters can be summarised briefly as follows; the sugar is puckered $\text{C}(3')\text{-endo}$ or $\text{C}(2')\text{-endo}$, the heterocycle is in an *anti* position about the glycosyl $\text{C}(1')\text{-N}$ bond, the orientation about the $\text{C}(4')\text{-C}(5')$ bond is *gauche*, *gauche* locating $\text{O}(5')$ 'above' the ribose and, finally, the phosphate group is *trans* to $\text{C}(4')$ (refs 21–23).

In Li^+NAD^+ the ribose bearing the nicotinamide residue is puckered in a $\text{C}(3')\text{-endo}$ envelope form while in the adenosine part the ribose occurs in a $\text{C}(2')\text{-endo}$ twist conformation, probably a consequence of the particular geometrical requirements imposed on the molecule by the coordination of Li^+ to both adenine N(7) and one free oxygen atom of the phosphate group P_N .

In both nucleotides the heterocycles are in an *anti* orientation with torsion angles $\text{O}(1')\text{-C}(1')\text{-N}(9)\text{-C}(8)$ of 52° for adenosine and $\text{O}(1')\text{-C}(1')\text{-N}(1)\text{-C}(6)$ of 15° for nicotinamide riboside. In the latter case, it seems that the positive charge at N(1) modifies the $\text{C}(1')\text{-N}$ bond distance, its value of $1.55 \text{ \AA}$ being significantly longer than the $1.47 \text{ \AA}$ generally observed in pyrimidine nucleosides, but it has no obvious influence on the nucleotide conformation. The orientations about the $\text{C}(4')\text{-C}(5')$ bond and about the $\text{C}(5')\text{-O}(5')$ bond in both nucleotides correspond to those usually considered as preferred, that is, *gauche*, *gauche* and *trans*, respectively.

As in other N(1) substituted nicotinamide moieties, the amide group in NAD^+ is nearly coplanar with the heterocycle, the C-NH_2 bond being *cis*-planar with the $\text{C}(3)\text{-C}(4)$ bond^{24–26}. In the unsubstituted nicotinamide²⁷, however, C-NH_2 is *trans*-oriented to $\text{C}(3)\text{-C}(4)$.

The pyrophosphate linkage

So far, four other crystal structures containing nucleotide pyrophosphate linkages have been reported, ATP, ADP, CDP and CDP-choline^{28–30}. Details of the pyrophosphate group have also been described for the symmetrical di- β -naphthyl-pyrophosphate³¹ and for thiamine-pyrophosphate³² and its hydrochloride³³. In all of these molecules the P-O distances in the P-O-P fragments differ significantly (more than three e.s.d.s) from each other. Although NAD^+ possesses a theoretical twofold symmetry about the pyrophosphate oxygen atom O(PP), the P-O(PP) distances differ greatly with a $\text{P}_\text{A}\text{-O(PP)}$ bond length of $1.56 \text{ \AA}$ and $\text{P}_\text{N}\text{-O(PP)}$ of $1.66 \text{ \AA}$. This is attributable to the asymmetrical Li^+ coordination. As with other pyrophosphates, the P-O-P angle is 133° and this relatively obtuse angle allows eclipsed conformation about the P-O(PP) bonds (torsion angles $\pm 120^\circ$) which are normally avoided. There is thus an appreciable flexibility about the pyrophosphate linkage as demonstrated by the almost evenly filled conformational wheel in Fig. 4. On the other hand, the torsion angle about the 'exo-pyrophosphate' link $\text{O}(5')\text{-P}$ is generally limited to (+) *gauche* with an exceptional -124° for the adenosine part in NAD^+ , probably a result of the steric peculiarities forced by the Li^+ coordination.

Li^+ cation coordination

One of the most interesting aspects of this crystal structure concerns the coordination of the Li^+ cation. The four tetrahedrally arranged ligands N(7), O(N1), O(N2), O(A1) (In this nomenclature, unesterified phosphate oxygen atoms

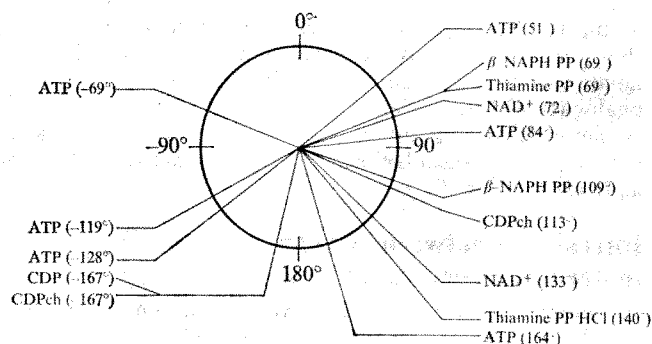


Fig. 4 Conformational wheel for torsion angles O-P-O-P about the pyrophosphate P-O linkage. Note that essentially all angles do occur except those with *cis*-planar arrangement (near 0°). CDPch, CDPcholine; β -Naph PP, di- β -naphthyl-pyrophosphate; thiamine PP, thiamine pyrophosphate, and thiamine PP-HCl, its hydrochloride derivative.

are indexed 1 and 2 if, looking along the $\text{O}(5')\text{-P}$ bond, they are at 120° and 240° clockwise rotation from the pyrophosphate link P-O(PP) are at distances of $2.13 \text{ \AA}$, $1.86 \text{ \AA}$, $1.92 \text{ \AA}$ and $1.88 \text{ \AA}$ from Li^+ respectively and are provided by two different NAD^+ molecules held together by the Li^+ cation in a head to tail orientation (Fig. 5). One NAD^+ molecule donates the two unesterified pyrophosphate oxygen atoms O(A1) and O(N1) and the other the adenine N(7) and the phosphate oxygen atom O(N2). A similar situation was found in the Na_2ATP complex²⁷ where the adenine N(7) atom is bridged with unesterified oxygen atoms of the γ -phosphate group, the coordination to the more remote γ -phosphate (instead of β -phosphate) probably reflecting the different radii of the cations: $0.68 \text{ \AA}$ for Li^+ against $0.97 \text{ \AA}$ for Na^+ . In this respect one might expect that Mg^{2+} with a radius of $0.66 \text{ \AA}$ would form cation-nucleotide complexes similar to those of Li^+ and not Na^+ or K^+ .

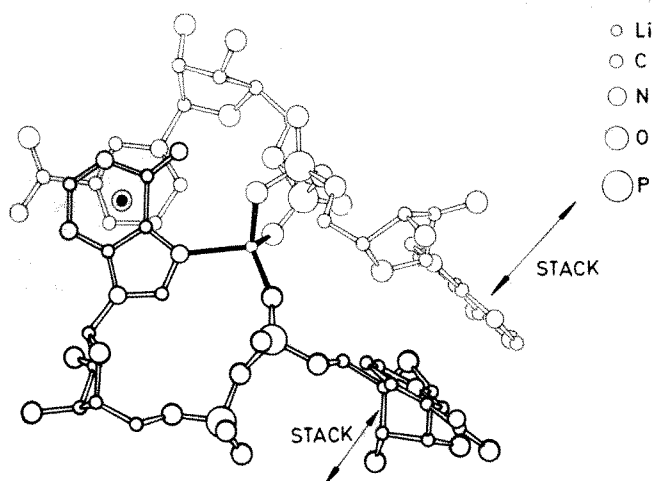


Fig. 5 The $\text{Li}^+ \cdot (\text{NAD}^+)_2$ dimer projected along the intermolecular stack indicated by $\odot$. Stack formation with other, symmetry related molecules in the crystal lattice are marked by arrows. One of the two NAD^+ molecules is drawn in heavier type for clarity.

Adenine and nicotinamide intermolecular stacking

Figure 5 demonstrates that the two NAD^+ molecules linked by one Li^+ cation interact through stack formation ($\sim 3.4 \text{ \AA}$) between the adenine coordinated to Li^+ and the partner nicotinamide. The partner adenine forms a stack with a symmetry related NAD^+ molecule to which it is bonded through another Li^+ bridge and so on. In this stack, the adenine N(6) amino group is close to the pyridine nitrogen atom, and the glycosyl linkages are oriented anti-parallel (Fig. 5), features also observed in the 1-methylnicotinamide-aden-9-yl acetate cocrystallite²⁶ but not in NAD^+ analogues with methylene chains replacing the ribotides^{34,35}. A similar arrangement of heterocycles was also proposed for the intramolecular stack in the 'folded' NAD^+ in aqueous solution^{7,10}.

Interaction between NAD^+ molecules in crystal lattice

As mentioned above, the NAD^+ molecules form Li^+ -coordinated, stacked 'dimers' which complex and stack with symmetry related neighbours, thus generating columns building up the crystal lattice. 'Empty' spaces between these columns are filled with two water molecules per NAD^+ , assisting in the formation of a complicated hydrogen bonding network (to be discussed elsewhere).

In aqueous solution, the 'polymeric' $\text{Li}^+ \cdot \text{NAD}^+$ complex will dissociate into electro-neutral $\text{Li}^+ \cdot \text{NAD}^+$ units with Li^+ coordinated intramolecularly either to N(7) and phosphate oxygen O(N2) or to the free pyrophosphate oxygen atoms O(A1) and O(N1), the remaining two coordination sites of Li^+ being satisfied by water molecules. Li^+ will be distributed between these two positions according to the respective association constants.

Conclusions

It is of particular interest to compare the structure of the $\text{Li}^+ \cdot \text{NAD}^+$ complex with those derived from the numerous spectroscopic investigations carried out in the past two decades and with the conformation of NAD^+ when it forms holoenzyme complexes with dehydrogenases.

Conformation of NAD^+ devoid of Li^+

The $\text{Li}^+ \cdot \text{NAD}^+$ complex represents only one possible low-energy structure of the NAD^+ molecule and with other

(larger) cations unable to coordinate with adenine N(7) and the P_N phosphate oxygen atoms simultaneously, geometries different to those observed here might be obtained. Some features of general importance will remain such as the preferred conformations of the two individual nucleotides but the orientation about the pyrophosphate linkage could change because of the flexibility about the $\text{P}-\text{O}(\text{PP})$ bonds as demonstrated in Fig. 4.

Correlation with results from spectroscopic data

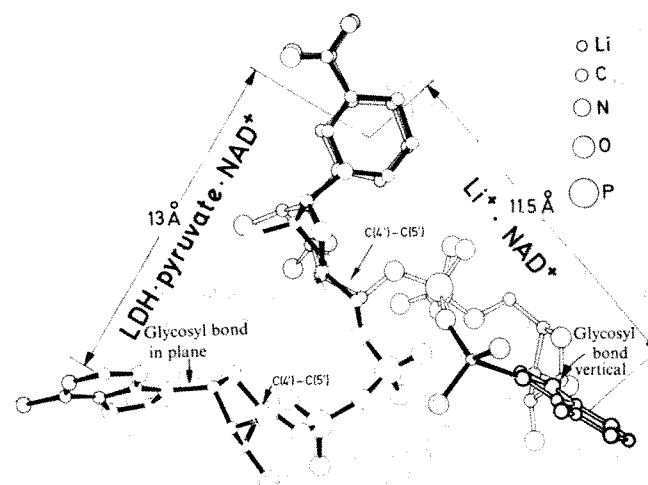
The most conclusive results on the conformation of NAD^+ in aqueous solution have been derived from NMR data. Although the proposal of two helical, 'folded' structures of NAD^+ was criticised in favour of the extended one¹¹, it seems to be corroborated by the numerous data. The helical geometry is even preserved when NAD^+ dimerises at higher concentrations¹⁰. From stereochemical considerations, a 'folded', helical structure for NAD^+ is feasible only if no chelation exists between adenine N(7) and the P_N phosphate group. Owing to the flexibility of the pyrophosphate linkage (Fig. 4), the respective torsion angles can be adjusted to form an intramolecular stack described in Fig. 5 for the $\text{Li}^+ \cdot \text{NAD}^+$ complex; N(6) of adenine is close to N(1) of nicotinamide and the glycosyl linkages are in nearly anti-parallel orientation. It should be noted that such a folding of the NAD^+ molecule can be achieved with the preferred conformations of both nucleotides fully maintained ($\text{C}(2') \text{ or } \text{C}(3')\text{-endo}$; *gauche*, *gauche*; *anti*).

In the light of the structure of the $\text{Li}^+ \cdot \text{NAD}^+$ complex with intermolecular chelation and dimer (stack) formation, however, it seems necessary to look at the interpretation of the spectroscopic data more critically, especially since in most previous work the nature of the counter ion has not been considered.

NAD^+ conformation on binding to enzymes

The crystal structures of enzyme· NAD^+ complexes have been described for LDH, LADH, GAPDH and s-MDH¹. In

Fig. 6 Structures of the NAD^+ molecules in the Li^+ complex (open bonds) and in the ternary complex LDH·pyruvate· NAD^+ ³⁷ (solid bonds; NAD^+ in the holoenzyme complexes has a similar conformation as in the ternary complex). Both molecules are projected on the plane through the nicotinamide heterocycle for easy comparison. Owing primarily to the different conformations about the $\text{C}(4')\text{--}\text{C}(5')$ bonds indicated by arrows, the adenosines are in different orientations relative to nicotinamide although the distances between the heterocycles are comparable. Note that the adenine planes are nearly perpendicular to the nicotinamide planes and that they are related by a 90° rotation: in $\text{Li}^+ \cdot \text{NAD}^+$ the glycosyl $\text{C}(1')\text{--}\text{N}$ bond is nearly vertical to the paper while it is in the paper for the ternary complex.



all of these enzymes, the nucleotide binding sites are geometrically related³⁶ and the bound NAD⁺ molecules display similar conformational features as shown in Fig. 6 for the ternary LDH-NAD⁺-pyruvate complex^{37,38}. It is striking to find that the intramolecular distances between the adenine and nicotinamide heterocycles, 11.5 Å in Li⁺-NAD⁺ and 13 Å in the ternary complex are comparable and that in both NAD⁺ molecules the conformation is such that the planes comprising adenine and nicotinamide are nearly perpendicular to each other. The form of NAD⁺ as found in the Li⁺ complex can therefore be considered as that form recognised by the enzyme before complex formation while the 'folded' conformation with parallel heterocycles about 3.4 Å apart seems to be too different to be directly involved in this process.

The conformations about the C(4')-C(5') bonds for NAD⁺ in the holoenzyme and Li⁺ complexes, however, constitute a significant difference. While for the Li⁺-NAD⁺ complex the preferred *gauche, gauche* conformation is found, it is *trans gauche*, for both nucleotides in the holoenzyme NAD⁺ molecule, corresponding to a high-energy state²³. This conformation has never been observed for any 5'-ribonucleotide except for the modified 6-azauridine-5'-phosphate (ref. 39).

Several factors can force such a drastic conformational change of a molecule, primarily the dehydration of NAD⁺ when diffusing from the aqueous solution to the enzyme surface. Further, the liganded cation will be expelled and replaced by positively charged groups of amino acid side chains lining the NAD⁺ binding site. These groups not only compensate for the positive charge but they can also form hydrogen bonds to phosphate oxygen atoms, to ribose hydroxyl groups and to the nicotinamide residue and, further, the adenine heterocycle fits into a 'hydrophobic' pocket^{1,36}. Together, these small individual forces can obviously induce a significant conformational change of NAD⁺.

Similarities of NADP⁺ and NAD⁺ conformations

NADP⁺ differs from NAD⁺ by an additional phosphate group attached to O(2') of the adenosine part. Because this phosphate is fairly remote from the pyrophosphate linkage, these two groups will not interact directly or by means of a chelated cation, at least as long as the two nucleotides

themselves maintain their preferred conformations. On the other hand, the adenine N(3) atom would be in a suitable position to be liganded by a cation connected with the oxygen atoms of the O(2')-phosphate.

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letters to nature

Evidence for a 581-d modulation in the pulse period of 3U0352 + 30

We have shown¹ that 3U0352+30 is an X-ray pulsator with a period of 13.9 min. There is also evidence for a modulation in the X-ray flux at 22 (or possibly 11) h but this phenomenon is less well-defined¹. The X-ray source has been identified with the 6 mag Be star X Per on the basis of position (ref. 2 and references therein) and interstellar absorption column³. Neither modulation has yet been detected in the broad-band optical flux^{4,5}, though there is unconfirmed evidence for a 7-min periodicity in a narrow band centred on 4,686 Å (ref. 6). Hutchings *et al.*⁷ have measured periodic shifts in the profiles of the Balmer and He I spectral lines of X Per with amplitudes of ~ 60 and 10 km s⁻¹ respectively which they interpret as due to orbital motion with a period of 581 d. They suggest that the Balmer

line shifts represent the underlying motion of X Per. This implies a mass for the unseen companion of tens of solar masses and indicates that it might be a black hole⁷. The necessity of reconciling all the X-ray and optical data has caused this interpretation to be questioned^{8,9}. Henrichs and Van den Heuvel⁸ suggest that the 581-d periodicity might reflect the apsidal motion of a neutron star in a 22-h orbit about X Per, while Milgrom⁹ postulates that the true 581-d orbital velocity is given by the He I absorption lines in the spectrum (velocity amplitude ~ 10 km s⁻¹), implying an unseen companion of only ~ 2M_⊙.

Copernicus has observed 3U0352+30 on a total of eight occasions between October 1972 and February 1977 as summarised in Table 1. We have searched five days of data from the observing run in February 1976 for a modulation in the pulsation period due to 22-h orbital motion. For a trial orbital period X the data were multiplied by a masking function F,

such that $F = 1$ for $0.0 < \phi_x < 0.33$ and $F = 0$ for $0.33 < \phi_x < 1.0$, where ϕ_x is the phase of the trial period. The 13.9 min. modulation period is then measured by power spectrum analysis¹. The mask is shifted by $0.16X$ and the process repeated until a complete cycle is covered. In this way an upper limit has been set to $V \sin i$ of 200 km s^{-1} for periods in the range 11–60 h. In Table 2 we give upper limits to the orbital inclination, i , for periods of 11.2, 22.4 and 44.8 h assuming a $20 M_\odot$ primary and a $2 M_\odot$ secondary in a circular orbit.

The mean heliocentric period of the 13.9 min modulation measured from each of the eight Copernicus observing runs is given in Table 1. The technique used to determine these values is as described in ref. 1 except that the uncertainties in the periods have been determined using the formula given by Middleditch and Nelson¹⁰. Numerical simulations on the data show that the confidence level of the uncertainties derived in this way is better than 99%. Table 1 shows that there are significant differences in the periods derived. We have investigated the possibility that these fluctuations are related to the 581-d optical period by fitting a function of the form

$$P = P_0 + P' t + A \sin(2\pi(t - t_0)/P_{\text{orb}})$$

where P_0 is the pulse period on JD 2441317.5 (0 h, 1st January 1972) and P_{orb} and t_0 are the period and epoch of a long term modulation. P_0 , P' , A , t_0 and P_{orb} were allowed to vary so as

Table 1 Observations of 3U0352+30 on eight occasions between October 1972 and February 1977

Observation start	Observation length (d)	Mean time of Observation (d) from 1 Jan 1972	Pulse period (min)
28 Oct 72	3.4	304	13.9330 $\pm$ 0.0038
22 Dec 72	1.9	358	13.9302 $\pm$ 0.0049
2 Feb 74	5.3	766	13.9315 $\pm$ 0.0019
10 Jan 75	10.8	1,111	13.9212 $\pm$ 0.0010
11 Feb 76	7.5	1,506	13.9229 $\pm$ 0.0015
12 Sep 76	15.4	1,724	13.9188 $\pm$ 0.0011
8 Jan 77	18.8	1,845	13.92097 $\pm$ 0.00070
4 Feb 77	12.6	1,868	13.9196 $\pm$ 0.0011

to minimise χ^2 . The best fitting value of A is found to be $0.0030 \pm 0.0008 \text{ min}$ ($V \sin i = 65 \pm 17 \text{ km s}^{-1}$), with the corresponding range of P_0 and P' being $13.9332 \pm 0.0002 \text{ min}$ and $-(2.72 \pm 0.07) \times 10^{-3} \text{ min yr}^{-1}$ respectively. The value of t_0 derived differed from that given by Hutchings *et al.* (JD 2442084) by $30 \pm 30 \text{ d}$ while, in the range 400–700 d, the optimum value of P_{orb} was $580 \pm 30 \text{ d}$. For the purposes of assigning confidence intervals^{12,13} to the parameters derived, the uncertainties given in Table 1 were conservatively treated as 1σ gaussian errors. Figure 1 shows the data with the best fit linear trend taken out and folded about a 581-d period. The solid line represents the best fit sine curve whose amplitude, A , is equivalent to a circular orbital velocity of $\sim 60 \text{ km s}^{-1}$.

Our data are thus consistent with a model in which the X-ray pulse period is modulated at the same period, phase and amplitude as the optical modulation found by Hutchings *et al.*¹¹ if an overall downward trend in pulse period is assumed. We consider this latter assumption to be justified since trends of this type are seen in other pulsating sources (for example

Table 2 Upper limits to the orbital inclination i for 3U0352+30

Period (h)	Predicted orbital velocity* (km s^{-1})	Upper limit to i from Copernicus data
11.2	763	15°
22.4	605	19°
44.8	480	25°

* Assuming a total mass of $22 M_\odot$.

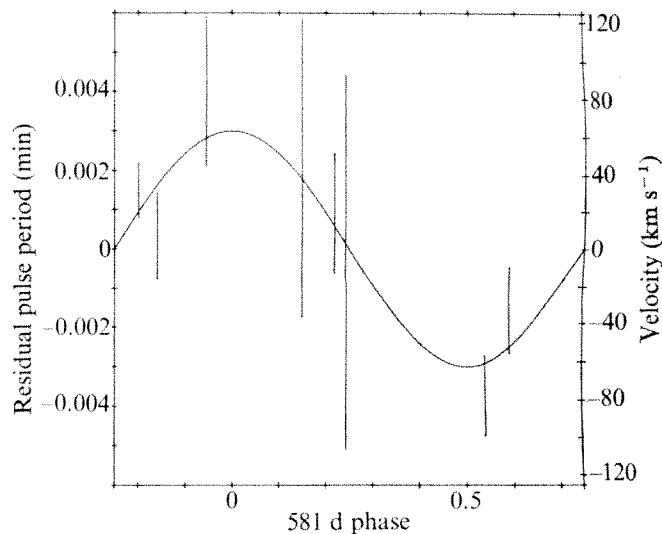


Fig. 1 The residual 13.9 min pulse period plotted as a function of the 581-d orbital phase. Phase zero represents X Per moving away from the observer at maximum velocity.

ref. 14). We recognise, however, that there are relatively few data, and it remains possible that we are observing (fortuitous) aperiodic fluctuations. More data are required over several 580-d cycles in order to bear out the effect. This will involve a substantial amount of effort because of the timescale of the phenomenon and the sensitivity required. Nevertheless we suggest that such an effort would be worthwhile since it could have important consequences for our understanding of the X Per system. In particular, if our present model of the data is correct we can make the following statements. (1) The secular decrease in period ($P/P \sim 10^{-4} \text{ yr}^{-1}$) suggests that 3U0352+30 is a spinning neutron star¹⁴ and not a white dwarf¹⁵. (2) The agreement in phase between the X-ray and optical modulations uniquely associates 3U0352+30 with X Per. (3) The amplitude of the X-ray modulation is consistent with the Balmer line shifts reported by Hutchings *et al.*¹¹. This is consistent with the premise of Hutchings *et al.* that these lines give the true orbital velocity of the system ($\sim 60 \text{ km s}^{-1}$) and lends support to the idea that this is a triple system with both X Per and 3U0352+30 orbiting an unseen companion with a mass of several tens that of the Sun.

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On the orientation of the pulsar space velocity vector relative to the spin axis

A SIGNIFICANT parameter for pulsars for which proper motion data are available is the angle ϕ between the space velocity vector and the spin axis. There are eleven such pulsars (ref. 15 and personal communication from one of the authors). The angle between the spin axis projected on the plane of the sky and the proper motion vector has a value close to 0° for three of them, close to 90° for six and between 0° and 90° for the remaining two. We present here a hypothesis which would explain how the different orientations of the space velocity vector relative to the spin axis arise. We distinguish between three modes of formation of pulsars and show that these correspond to the three different sets of values of the angle.

The supernova explosion of a rotating magnetised star would result in the ejection of double jets along the spin axis¹, and only a part of the stored magnetic energy is sufficient to eject the supernova shell². Since in real stars the field is usually not at all symmetrical between the two poles, one would expect the jet ejection to be an asymmetric process resulting in the neutron star getting a kick along the spin axis. Asymmetric radiation, due to the dipole being off centre, from the resulting pulsar could give it an additional kick along the spin axis³. In other words, pulsars formed from the supernova explosions of single stars should have space velocities along the spin axis; pulsars with $\phi \approx 0^\circ$ (0531+21, 0833-45 and 2016+28) belong to this category.

For a supernova explosion to take place in a close binary system, the primary should have a mass $\geq 15M_\odot$ (ref. 4). The primary evolves quickly, transferring more than two thirds of its mass to the companion before it explodes as a supernova, leaving behind a relativistic star: a neutron star or a black hole. Since the more evolved component is also the lighter (at the time of the explosion), mass ejection will not disrupt the binary⁵.

Two other mechanisms are available in principle for disrupting the binary. (1) The ejected supernova shell could impart a critical impulse to the unexploded companion making it leave the system⁶. It has been shown⁷, however, that this mechanism is not likely to be effective. (2) A highly asymmetric supernova explosion could impart a sufficient kick to the exploding star to push it into a hyperbolic orbit. To disrupt the binary in this manner, the impulse should be imparted in the direction of the orbital motion⁸. It is extremely unlikely that a kick normal to the orbital plane—assuming of course that the spin axis is perpendicular to the orbital plane—would disrupt the system. Highest observed space velocity for single pulsars is around 400 km s^{-1} (ref. 9). Velocities of this order are definitely unable to disrupt a binary system especially if the kick is normal to the orbital plane. So the first supernova explosion leaving behind a relativistic star core is unlikely to disrupt the binary. However, to account for the existence of runaway stars, it is necessary to disrupt some of the binaries at this stage. It should therefore be conceded that some supernova explosions do not leave behind any core¹⁰.

If a neutron star were formed in a massive binary it might tilt the orbital plane and impart a velocity of a few dozens of kilometres per second to the centre of the mass of the binary. It could also make the orbit eccentric but this would eventually become circular due to tidal action forcing the OB type main sequence star into synchronous rotation¹¹. The binary would manifest itself as an X-ray source as long as mass being accreted by the compact star from the OB type star (now a blue supergiant) is large enough to give a measurable X-ray flux¹², but not too large to choke the source¹³. Large mass loss would ensue when the primary evolves beyond the blue supergiant stage and overflows the Roche lobe. The X-ray source would be extinguished and matter start flowing out from the outer Lagrangian point. Enhanced accretion by the neutron star and mass loss from the system would spin up the neutron star¹⁴.

The explosion of the resulting helium star is likely to disrupt the system because it is more massive than the companion neutron star. (If the companion is a black hole, disruption probability would be smaller because then both the members of the system will have comparable masses). The disruption of the orbit would release two neutron stars. The old, reactivated neutron star would move in the orbital plane with its orbital velocity. So it would have a space velocity normal to the spin axis¹⁵. This is how pulsars with $\phi \approx 90^\circ$ (0823+26, 0834+06, 1133+16, 1237+25, 1929+10 and 2021+51) were liberated from binary orbits which were disrupted by the supernova explosions of the companion stars. The exploding star would get a kick along the spin axis¹ and so it will move with a velocity equal to the vector sum of the mutually perpendicular kick velocity and its orbital velocity. Pulsars with $0 < \phi < 90^\circ$ (0329+54 and 0950+08) are thus produced by the supernova explosions in and leading to the disruption of the binary systems.

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How did barium titanate particulates stick together in the nebula?

TANAKA *et al.*¹ have suggested that there must be barium-rich micro-components in the Allende meteorite. We have confirmed this prediction by discovery of barium-rich particulates (see ref. 2). Figure 1 shows diffuse distribution of Ba-rich particles as well as their clusters; each of apparent particles seems to be composed of finer particulates probably of submicron size. Tanaka and Okumura³ showed that the Ba-rich spots are also high in content of titanium. Figure 2 shows the areal overlapping of the distribution of Ba with that of Ti. Relatively sparse dots for Ti are due to shorter scanning time than for Ba.

The results of electron microprobe studies² show the atomic ratio of Ba to Ti to be almost 1:1. Moreover, based on the data obtained by Tanaka and Okumura³, contents of BaO and TiO₂ in most Ba-Ti-rich portion turn out to be 59 and 34% respectively; the pure chemical formula BaTiO₃ gives the theoretical values 66% BaO and 34% TiO₂. Thus the purity of micro-component as barium titanate is amazingly high. The small difference between actual and theoretical contents may be due either to experimental errors or to the presence of small amounts of other titanates and probably silicates. Palme and Wlotzka⁴ separated a particle (about $20 \mu\text{m}$) rich in refractory siderophile elements Os, W, Re, Ir, Mo, Ru and Pt from the Allende meteorite.

The Ba content of the Allende BaTiO₃ is therefore up to 1.7×10^4 times the corresponding chondritic abundance.

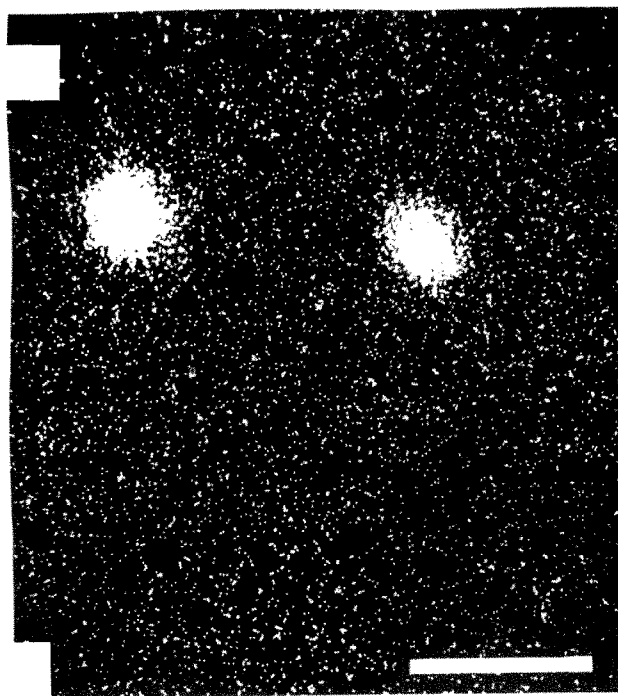
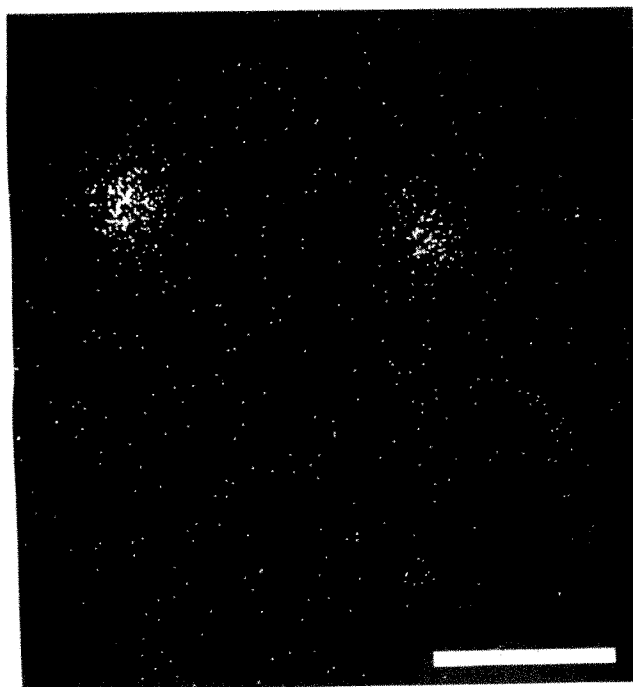


Fig. 1 Distribution of Ba microconcentrates in the matrix of Allende; scale bar indicates 10 μm .

This occurrence of barium titanate can be compared with that of calcium titanate (CaTiO_3), which occurs as perovskite in carbonaceous chondrite^{4,5}. According to Grossman⁶, the condensation temperature of perovskite is 1,647 K at 10^{-3} atm total pressure, implying that this mineral is one of the highest-temperature minerals to be condensed early in the solar nebula. It is important to know whether the condensation temperature of BaTiO_3 is higher or lower than that of CaTiO_3 and what the temperature difference is, but the condensation temperature of BaTiO_3 is not

Fig. 2 Distribution of Ti microconcentrates in the matrix of Allende; scale bar indicates 10 μm .



quantitatively known. It can be assumed from analogy, however, that this compound also has a high condensation temperature.

The Ba abundance⁷ in ordinary chondrites is between 3 and 4 p.p.m. The corresponding abundance⁸ of Sr is around 10–12 p.p.m., so the atomic abundance ratio of Sr to Ba is about 5. We might therefore expect the occurrence of SrTiO_3 in the same sample. Despite the careful X-ray scanning, however, it is significant that we have failed to detect the concentrates of Sr like those of Ba. Even apart from this observation, it is difficult to see how the barium titanate particulates could selectively stick to each other in the nebula. This is of even more interest when the low cosmic abundance of Ba is considered. Thus, is it conceivable that some special factor was operative in this selective sticking of BaTiO_3 particulates? Here we propose that the following unique properties of BaTiO_3 are associated directly with the formation of barium titanate particulates in these conditions.

Barium titanate has five crystalline modifications⁹: cubic, tetragonal, orthorhombic, trigonal, and hexagonal. Except for the hexagonal form which is stable above 1,733 K, other forms are well known for their highly dielectric and ferroelectric properties, while the dielectric constant is a function of temperature¹⁰. In particular, the tetragonal form which is stable between 393 and 278 K is known for being piezoelectric and ferroelectric. The Curie temperature is 393 K.

These physical properties imply that barium titanate fine particulates are liable uniquely to high dielectric polarisation in the presence of electric field and the electric charge is raised on their surfaces. It would be understandable that electrostatic situation like this is very favourable for selective attraction of BaTiO_3 fine particulates into larger particulate or aggregate. If BaTiO_3 particulates had still been floating in the nebula at the Curie temperature (393 K), the selective sticking of BaTiO_3 must have been promoted especially at this temperature (ref. 10). The electric field may have been prevailing due to paleosolar wind¹¹ or to friction of dust grains themselves, although other sources of electric field might be conceivable. It should be noted, however, that even without an electric field, spontaneous polarisation can arise for ferroelectric substance.

This reasoning in terms of electrical characteristics of BaTiO_3 seems to be parallel with the observation by Tanaka and Okumura²; the relatively large cluster of Ba–Ti-rich particles is found in matrix of meteorite rather than in inclusion composed seemingly of higher-temperature minerals. From the presence of magnetite in matrix of Allende, Larimer and Anders¹² estimated the upper limit of 400 K for accretion temperature of matrix. Consideration of the reaction of olivine with water vapour to form hydrous silicates led Anders¹³ to the reaction temperature range of 300–350 K. It should be noted that these numerical values possibly indicating the accretion temperatures for matrix are close to the Curie temperature of BaTiO_3 . (This does not conflict with our understanding that this compound was originally formed at high temperatures.)

As mentioned above, unlike Ba, the concentrates of Sr have not been detected in spite of the chemical similarity between both elements as alkaline earths. One of the plausible explanations of this fact is that the difference in ionic radius between Ca and Sr is smaller than between Ca and Ba, and Sr may therefore be more readily incorporated into calcium minerals. Alternatively, the relevant difference of Sr from Ba may be that the dielectric constant of SrTiO_3 is an order of magnitude lower than that of BaTiO_3 and, in addition, that the Curie temperature of the former is about 70 K, compared with about 400 K for the latter. These two possible explanations may not always be alternatives, but may be cooperative factors.

Discovery of pure ultra-fine particulates of BaTiO_3 opens new aspects in meteoritics. As maintained above, special

attention should be given to the unique electric properties of BaTiO₃. The very wide variation of Ba contents¹ must also be considered—the Allende meteorite does not represent the whole range of products which formed in a closed space box containing every element in proportion to cosmic abundances. Components of strange origin, even if solar, are certainly added in excess to the 'closed-space-box' products.

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Rates of reaction of HO₂ with HO and O studied by laser magnetic resonance

CONCERN about the depletion of stratospheric ozone by nitrogen oxides released by high flying aircraft¹ and by chlorine species from the decomposition of halocarbons^{2,3} has emphasised the need for laboratory data on the elementary reaction steps involved, particularly those which control the concentration of the hydroxyl radical (HO) in the stratosphere. HO has an important role in both ozone destruction mechanisms, since it combines with NO₂ to form the relatively inert HNO₃ molecule and converts the inactive HCl formed from Cl+CH₄ back into active Cl atoms. In the stratosphere the 'odd hydrogen' species H, HO, and HO₂ are formed by the reactions of O(¹D) with H₂O, H₂ and CH₄ and by the photolysis of H₂O, processes which are comparatively well understood. The odd hydrogen species are interconverted largely by reactions such as



and removed mainly by



The rate coefficients for reactions (1) to (3) are relatively well established^{4–6} but there is no previous experimental determination of k_4 and measurements of k_5 (which have not been based on direct and unequivocal measurements of the concentration of HO₂) have yielded values between 2×10^{-11} and 2×10^{-10} cm³ molecule⁻¹ s⁻¹ (refs 7–9). We report the first direct measurements of the rate coefficients k_4 and k_5 for which we have used the novel technique of laser magnetic resonance.

HO and HO₂ are detected in a fast flow system at total pressures of a few torr and 293 K by using a strong magnetic

field to bring Zeeman components of their ²Π_{3/2}, ⁵/₂ ← ⁵/₂ and 4_{3,2} ← 5_{2,3} rotational transitions into resonance with the 118.6-μm line of a water vapour laser. The molecules are detected within the laser cavity and a thin, rotatable polyethylene Brewster window separates the laser discharge from the gas flow system (Fig. 1). The applied magnetic field is weakly modulated at 35 Hz and signals from a Golay cell, which monitors the laser output through a small hole in one mirror, are passed through a phase sensitive detector system and displayed in derivative form as in EPR. The lines show both Doppler and pressure broadening and, in the case of HO, can exhibit modulation broadening. The method of moments is therefore used to convert the differential traces into integrated absorptions from which the ratio of concentrations of HO₂ and HO can be calculated by using the matrix elements and *g*-factors for the transitions and the rotational partition functions^{10,11} and the dipole moments of the two species (S. D. Peyerimhoff, personal communication, refs 12 and 13 and G. Gillespie quoted in ref. 14).

Absolute sensitivities were established by generating known concentrations of HO using the rapid stoichiometric reaction



The reaction between HO and HO₂ has been investigated by studying three related chemical systems; in each system a stationary state in [HO₂] is established by reactions (7) and (5)



Here measurement of the absolute concentration of HO₂, together with the value of $k_7 = 7.9 \times 10^{-13}$ cm³ molecule⁻¹ s⁻¹ at 293 K (ref. 15) leads to a value for k_5 .

Possibly the simplest system is the reaction of H₂O₂ with HO which has previously been generated in reaction (6) conducted in the presence of a small excess of NO₂ over H atoms. Reaction (6), however, produces equal amounts of NO and HO; with the relatively long residence times used (~70 ms) the decay of HO on the walls ($k_w = 25 \pm 5$ s⁻¹) reduces the concentration of HO below that of NO at the point of H₂O₂ addition. Since reaction (8)



might cause errors in the analysis, other systems which avoid this complication were also investigated. In these, HO and HO₂ are generated by the reaction of H atoms with H₂O₂ and subsequently reactions (7), (5) and (9)



largely determine their concentrations. This reaction was allowed to proceed for about 30 ms and then sufficient NO₂ or O₃ was added to scavenge all the remaining H atoms with concomitant production of HO. Here the ratio of NO to HO is clearly unity if NO₂ is the scavenger and NO is absent when O₃ is used. The concentration of HO₂ present is then essentially governed only by reactions (7) and (5) and can thus be used to deduce k_5 . The central values of k_5 obtained from each type of experiment differed by less than the errors associated with the measurement of flow rates, signal strengths and so on, implying that nitrogen oxides do not interfere significantly in our conditions. The average value found for k_5 was $5.1 \pm 1.6 \times 10^{-11}$ cm³ molecule⁻¹ s⁻¹ at 293 K.

The reaction O+HO₂ was investigated in the reaction system

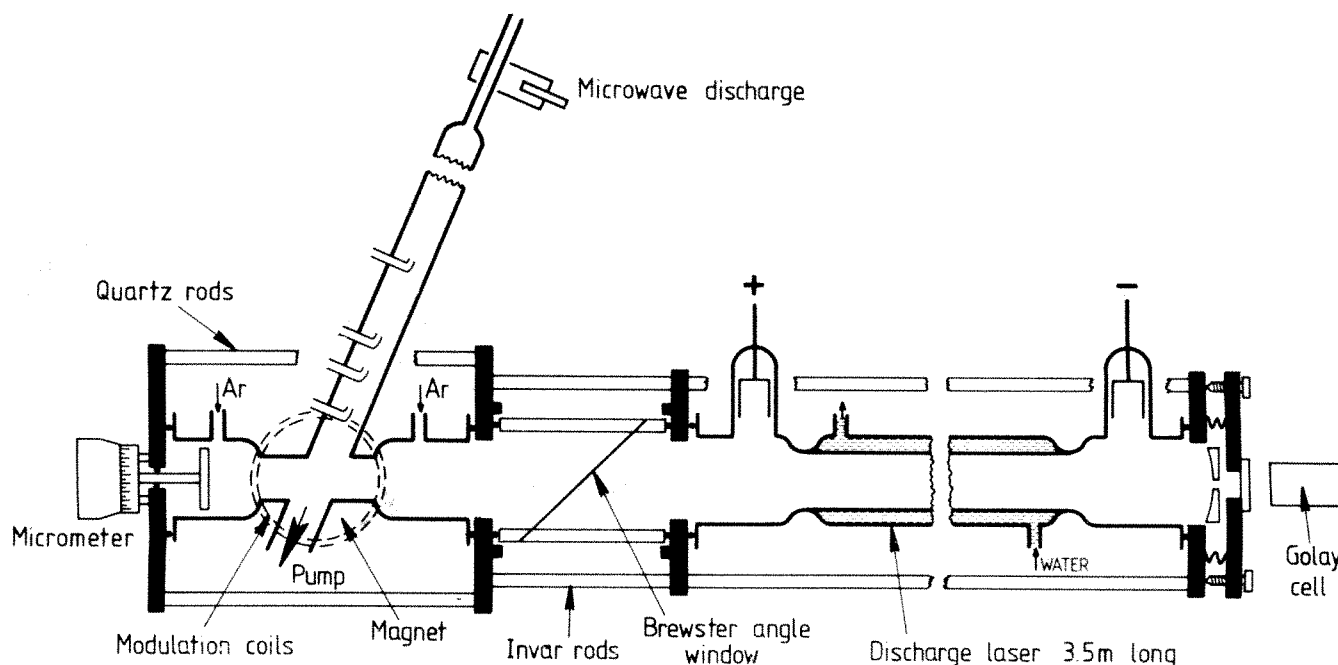
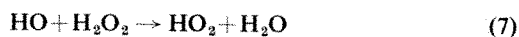
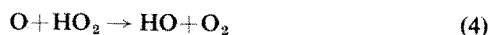
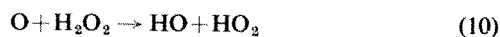


Fig. 1 Laser magnetic resonance spectrometer for kinetic studies.

O atoms plus H_2O_2 which is described by the following elementary reaction steps



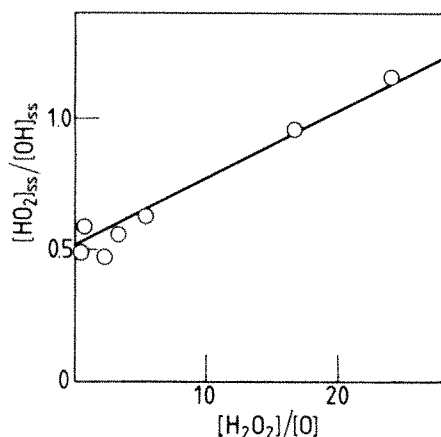
and the relative stationary state concentrations of HO_2 and HO are given by

$$\frac{[\text{HO}_2]}{[\text{HO}]} = \frac{k_3}{2k_4} + \frac{k_7}{k_4} \frac{[\text{H}_2\text{O}_2]}{[\text{O}]}$$

The ratio of the slope to intercept of a plot of this relationship (Fig. 2) is 4.7×10^{-2} which agrees very well with the literature value of the ratio $2k_7/k_3$ (refs 5,15) providing confirmation of the above mechanism. The average value of k_4 obtained from the slopes and intercepts of such plots is $k_4 = 3.53 \pm 1.0 \times 10^{-11} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ at 293 K.

The values of k_4 and k_5 are close to the expected pre-exponential factors for such reactions and the values should apply at stratospheric temperatures within the present accuracy of our measurements.

Fig. 2 Steady-state radical concentrations in $\text{O} + \text{H}_2\text{O}_2$ reaction.



A further interesting point is that the calculated concentrations of HO radicals in the middle and upper stratosphere are roughly proportional to $(k_4/k_5)^{1/2}$ and this ratio would not be affected by any inaccuracy in the calibration for HO_2 since both rate constants would be affected in a similar way.

Measurements of the rate coefficients of the $\text{H} + \text{HO}_2$ reactions and of the temperature coefficients of these processes are under way and the full details of these measurements will be described elsewhere.

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A new classification of metal catalysts in skeletal reactions of hydrocarbons

GENERAL knowledge about the factors governing the catalytic activity of various metals in different skeletal reactions of hydrocarbons is rather limited. Comparative studies are reported here on the depth of hydrogenolysis and the formation of saturated products of 3-methylpentane (3MP) and methylcyclopentane (MCP) for eleven different

metals. We have found that isomerisation, C_5 ring closure and opening activity of Rh, Pd, Ir and Pt can be correlated with their f.c.c. structure, and their atomic dimensions being in a narrow range. These four metals promote single hydrogenolysis whereas multiple fragmentation is the only reaction observed over Co, Ni, Cu, Ru, Ag, Re, Os. Hydrogenolysis activity and pattern are less characteristic for each group.

Various aspects of metal catalysed skeletal hydrocarbon reactions have been studied recently¹ to give some classification of various catalysts. For example, the depth of hydrogenolysis has been found higher over non-noble (Fe, Co, Ni) than over noble VIIIB group metals²⁻³; Re (and even Os) promote deep hydrogenolysis, too⁶. Cyclopentanic C-C bonds have been reported to be similar to alkanes in their resistance to hydrogenolysis¹, although at least two mechanisms have been proposed for ring hydrogenolysis^{7,8}. Two mechanisms of skeletal isomerisation ('bond shift' and ' C_5 -cyclic') have been found over Pt (ref. 1); Pd, Rh, Ir, Ru have also been reported to isomerise *n*-heptane⁹. Hydrogenolysis has been described as a structure-sensitive reaction¹⁻³, and crystallite size effects may also influence the mechanisms of isomerisation¹.

We intended to compare various metals in conditions at which their catalytic activity is as close to each other as possible. Data in Table 1 are related to selected temperatures corresponding to nearly equal rates of 3MP hydrogenolysis. Hydrogenolysis activity is inversely proportional to the τ values. Crystallite size effects between 'powders' and 'blacks' are indicated by the fact that higher τ values are required in the former case to ensure identical activity per unit of surface. The depth of hydrogenolysis can be characterised by a fragmentation factor ζ . This is defined as the number of fragment molecules per molecule of hydrocarbon hydrogenolysed. This value is apparently independent of the structure of the initial hydrocarbon, the origin of the catalyst (that is, of its crystallite size) and the actual τ values at a given conversion level and given hydrogen pressure. C_6 product formation is characterised by the ratio of alkane isomers.

The metals studied seem to fall into two different groups. Group A contains four noble VIIIB metals—Rh, Pd, Ir, Pt—with ζ values near to two which means that single rupture of the molecule predominates. They promote isomerisation and C_5 -cyclisation of 3MP and produce very

high amounts of C_6 alkanes from MCP without altering the fragmentation yields and ζ values as compared with 3MP. On the other hand, the product spectrum over Group B metals including Co, Ni, Ru, Re, Os (and also Cu and Ag where τ was not reached) consists of fragments only except for Ru which produces traces of C_6 isomers. The ζ values between 3 and 6 indicate the multiple breakdown of the molecules. C_6 -alkanes represent a minor fraction of the product spectrum from MCP, their formation being apparently a part of the general scheme of molecular breakdown.

2MP/nH ratios show that alkane production from 3MP and MCP should be related processes; these ' C_5 -cyclic reactions' (including C_5 ring closure, opening and their combination: C_5 -cyclic isomerisation) are confined to group A metals. The higher rate, lower energy activation and different selectivity of ring opening indicates that it represents a particular type of C-C bond rupture distinct from either single or multiple alkane hydrogenolysis. Obviously, the difference can be attributed rather to the nature of the metal than to the structure of MCP¹⁰.

The pathways of cyclohexane dehydrogenation (through flatly or edgewise chemisorbed rings) have been correlated with the catalyst surface geometry, group A metals being the flatly adsorbing ones¹¹. These metals (and only they) have f.c.c. lattice with interatomic distances between 2.69 and 2.77 Å (ref. 12). With such a geometry, the spacing between interatomic holes on the closest packed (111) plane is appropriate (Rh: 1.55 Å Pt: 1.61 Å) for accommodating neighbouring carbon atoms of a hydrocarbon (C-C bonds between 1.522 and 1.546 Å (ref. 13)) over these sites. Such surface intermediates are suggested for single hydrogenolysis without disputing ideas about their nature or dissociated character¹⁻³. From Pt to Rh the ζ value remains almost constant (Table 1); with other surface geometry, the sharply increasing ζ values indicate that multiple hydrogenolysis should involve other surface species. We tentatively suggest that these are bonded to the top of metal atoms.

The concept of flatly chemisorbed C_5 -cyclic intermediate (suggested for C_5 -cyclisation over Pt (ref. 14)), can be extended for every C_5 -cyclic reaction over each group A metal. One active site may serve as a binding site (interacting preferably with the tertiary carbon atom), the other acting as a catalytic site for ring opening and/or closure.

Table 1 Comparison of 3-methylpentane and methylcyclopentane reactions over various metals

Metal	τ (K)	3 MP			2MP nH	MCP			2MP nH
		< C_6 §	ζ	C_6 § ¶		< C_6 §	ζ	C_6 § **	
Co*	573	1107	4.99	0	—	3060	4.98	130	1.9
Ni*	543	463	3.13	0	—	380	3.11	120	2.8
Cu†	663	134	2.24	0	—	—	—	—	—
Ru*	513	545	3.76	0	—	1070	3.82	130	0.2
Ru†	543	544	3.71	3.3	~2	520	3.46	40	1.7
Rh*	513	591	1.86	34	—	75	2	655	1.6
Rh†	453	1100	1.80	0	—	550	1.78	6600	6.5
	498††	15450	2.17	330	5.6	11100	2.10	7460	6.5
Pd*	588	813	2.01	578	6.5	—	—	—	—
Pd†	648	610	2.08	1080	3.4	280	2.48	1630	2.9
Ag†	723	22	2.55	0	—	—	—	—	—
Re*	633	805	3.45	0	—	690	3.37	120	0
Re†	573	782	3.35	0	—	1070	3.12	110	0
Os*	513	455	5.91	0	—	2070	5.97	90	0.5
Ir*	543	1000	1.92	410	6.4	1000	1.81	5900	5.7
Ir†	468	1060	1.76	430	—	1300	1.70	23600	32.4
Pt†	663	278	2.09	980	4.9	—	—	—	—
Pt††	603	482	1.89	2420	5.7	320	2.14	9600	9.8

Pulse-microcatalytic system; strictly identical 3- μ l pulses introduced into 60-ml-min⁻¹ pure hydrogen carrier gas stream.

*Commercial powder. †Precipitated black. ‡Low surface sintered precipitated metal. §Expressed as 10¹⁵ C_6 molecule reacted per m² metal.

¶Values lower than 2 may be due either to error in calculation or to actual methane deficit indicating irreversible adsorption. ¶Isomer C_6 alkanes (2MP, nH, eventually 2,2 DMB) and MCP. ** C_6 alkanes (2MP, 3MP, nH). ††Temperature for maximum isomerisation yield.

Such species also require definite surface geometry: with spacings that are too large (e.g. Ag) the chain ends cannot come together; with decreasing atomic diameter, the relative importance of hydrogenolysis as against isomerisation increases (cf. Table I), indicating that the attraction of next nearest neighbour sites from smaller and smaller distances gradually suppress C₅-cyclic reactions and promotes hydrogenolysis.

This concept is in agreement with the electronic inequality of tetrahedral (e_g) and octahedral (t_{2g}) sites¹³; it may also explain both the necessity of hydrogen for C₅-cyclic reactions¹⁴ and the selectivity (Fig. 1).

There are two possible explanations for c.p.h. Re and Os with proper atomic dimensions belonging to group B, in spite of the similarity of f.c.c. (111) and c.p.h. (0001) faces. The probability of occurrence of analogous crystallographic planes may not be equal¹²—higher hydrogenolysing activity on c.p.h. Ru and Os was explained this way¹⁶. Or the discrepancy may underline the role of sites where the geometry is favourable on f.c.c. metals only (e.g. steps or B₅ sites¹⁷) (Fig. 1).

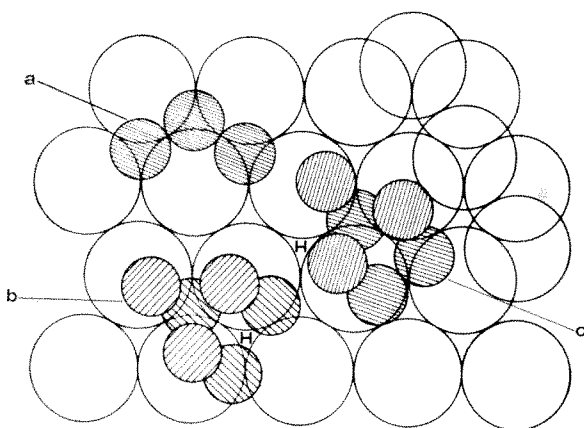


Fig. 1 Possible geometry of chemisorption over platinum (111) plane. The dimension of metal (larger circles) and carbon (smaller shaded circles) atoms is to scale. *a*, 1, 2, 3-chemisorption with participation of both e_g and t_{2g} sites leading to hydrogenolysis (1, 2- or 1,3-sorption is also possible); *b*, 3MP chemisorption over two neighbouring t_{2g} site, with hydrogen atoms blocking more active e_g sites; *c*, one of the possible accommodations of MCP along a step [exposing (311) sites^{18,19}] ring opening over the B₅ site would give 2MP, over the neighbouring t_{2g} site 3MP; *n*-hexane formation is hindered.

We do not want to suggest that surface geometry is the only influence: the crystal parameters are outward manifestations of the collective interactions between individual atoms, but it should be pointed out that surface electronic inhomogeneity may create active sites the geometry of which may also be important in catalysis.

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Surface wind and sea waves in a hurricane field

THE hazards associated with a hurricane are extremely strong wind, violent seas and heavy rain. Over the seas, heavy rain does not pose as much problem as the other two. It is very rare to get detailed information of surface winds and waves in the hurricane field over the seas. The only set of documented data regarding surface wind and heights of waves is by Arakawa and Suda¹ describing the experience of main squadron of the Imperial Japanese Fleet as it inadvertently passed through the typhoon of September 1935. These simultaneous observations of weather and sea conditions are some of the best data on record. The hurricane reconnaissance over the Atlantic and Pacific could give the structure of winds in the hurricane field at 1000 ft or more above sea level, but no detailed observations about surface winds and waves are available.

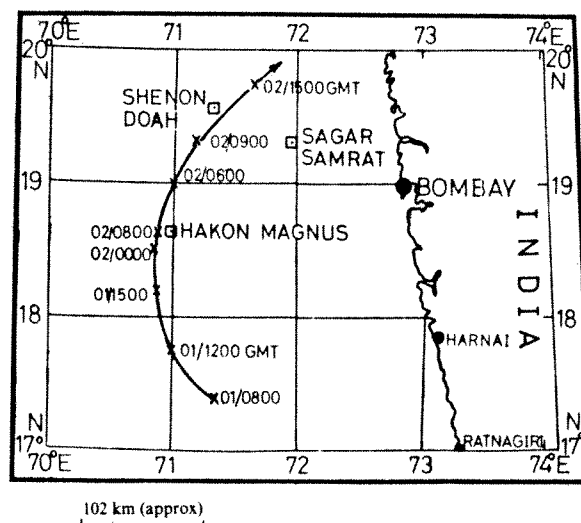


Fig. 1 Cyclone tracking by radar fixes of storm in Arabian Sea 30 May-3 June 1976, showing position of drilling rigs.

During the period 31 May-3 June, 1976, a cyclonic storm passed over Arabian Sea. On 2 June it reached hurricane stage and passed through Bombay High Area where off-shore oil explorations are being conducted by the Indian Government. Fig. 1 gives the track of the storm with the positions of three jacked-up rigs: Haakon Magnus, Shenan Doah and Sagar Samrat. The storm centres referred to here are derived from the radar observations at the Area Cyclone Warning Centre, Bombay. It is a 10-cm radar with an effective range of 400 km. The rigs continuously recorded observations of wind and wave. The wind observations were made by standard wind instruments installed at the rigs and the wave measurements were taken with reference to the markings on the legs. These wave

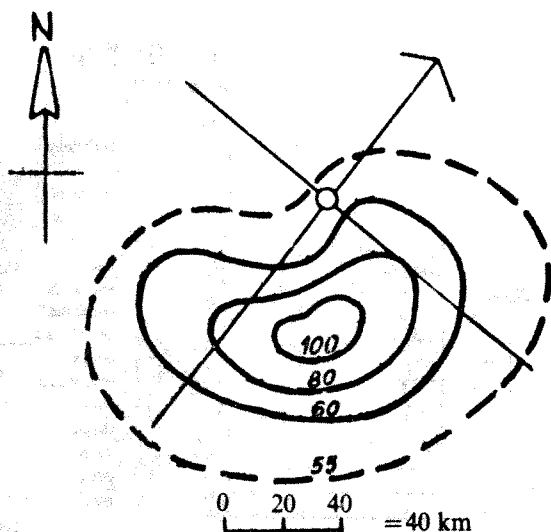


Fig. 2 Wind field over storm area, showing wind speed in knots. The arrow shows the direction in which the storm is moving.

observations are therefore taken to be more accurate than could be done on board a ship.

A composite wind speed chart was plotted throughout the day on 2 June. There was little variation with time: and the hurricane strength was maintained. The isotachs were drawn on this composite chart to determine the distribution of wind speed over different sides of the storm. This is shown in Fig. 2. It is seen that the strongest winds are found in the right rear quadrant and is very similar to the one found by Hughes² from aircraft reconnaissance of tropical storms over Pacific Ocean. Our observations differ from those of Arakawa who found the core of strong winds both in front and rear quadrants on the right side.

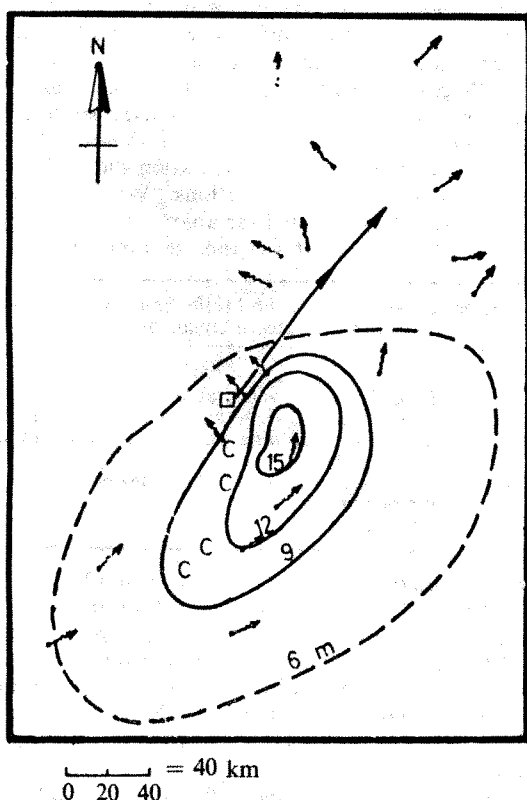


Fig. 3 Composite chart showing wave heights and directions. C indicates confused direction.

The composite chart for wind direction was also plotted (figure not presented). It was seen that the centre of the storm as determined by radar did not agree exactly with the convergence centre of wind pattern. The difference was found to be 20 km, the wind centre being to the right of radar centre.

The composite chart for wave heights and directions is shown in Fig. 3. The pattern is similar to one found by Arakawa for the sea in a typhoon, except that we found the highest waves both in the front and rear quadrants of the right side, whereas they found them in the right rear quadrant only. Wave directions generally agree with the theoretical model³. The sea was confused from the centre to the right rear quadrant.

The periods of waves varied between 6 and 12 s. The wave energy is given by $E = 41 H^2 T^2$ ft lb per foot of wave crest per wave length⁴. The maximum energies of waves encountered by the rigs were calculated (see Table 1).

Table 1 Maximum energies of waves encountered by rigs

Rig	Wave energy, E (10^6 J)
Sagar Samrat	1.72
Haakon Magnus	5.01
Shenan Doah	1.18

Energy values are given per metre of wave crests per wave length.

Assuming that the energy of wave is due to transfer of kinetic energy from air-water-surface interaction *in situ*, $E_{\max}$ is expected near the centre of the storm. This is confirmed by the value we get for Haakon Magnus.

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Imbricate thrust model of the Southern Uplands of Scotland

FAUNAL and structural evidence^{1–3} suggest that a wide ocean was present between the Southern Uplands of Scotland and the English Lake District, and that during the late Ordovician and the Silurian the Southern Uplands were situated on the continental margin of North America, where the Iapetus Ocean was being subducted. Here we explain the stratigraphical and structural relationships observed in the Southern Uplands by a dynamic model involving sequential accretion of an imbricating sedimentary wedge on to the continental margin. This evolutionary pattern is analogous to those suggested for many modern convergent plate boundaries^{4–15}.

The Southern Uplands has been divided stratigraphically into three units¹⁶ (Fig. 1). The Southern Belt is composed of Wenlock greywackes and shales, some of which contain graptolites¹⁷. The Central Belt consists of greywackes overlying condensed Moffat Shale successions of mid-Ordovician to Llandovery shales, ashes and cherts. In the

Northern Belt, which is entirely of Ordovician age, mafic volcanics, bedded cherts and, in some places, graptolitic shales underlie greywackes. The shales provided Lapworth with the data for his classic work on the use of graptolites as stratigraphic indicators; the seventeen zones of the Upper Ordovician and Lower Silurian have an average thickness of less than 5 m when present in this facies (see Fig. 2).

The general succession resembles many to be found in ocean basins today¹⁸, except that calcareous plankton were absent during the Lower Palaeozoic, and carbonaceous graptolitic shales occupy the position held by calcareous oozes in many modern oceanic sequences.

The rocks in the Southern Uplands have been interpreted as oceanic or trench deposits in the majority of previous plate tectonic reconstructions^{1,2,4,19}, but contrary views have been expressed based on the presence of high velocity rocks at depths of around 15 km below the Southern Uplands²⁰. On close inspection, however, the phase velocities recorded at the Eskdalemuir seismic array²¹ show considerable scatter, but there is a tendency for fast and slow-velocity source positions to be controlled by regional (ENE–WSW) strike. The slow velocities are obtained from positions in directions transverse to strike, and the fast velocities come from directions parallel to strike. It seems possible, therefore, that there is no sharp contact between low and high velocity rocks, and the higher velocities could be derived from metamorphosed Lower Palaeozoic sediments at depth.

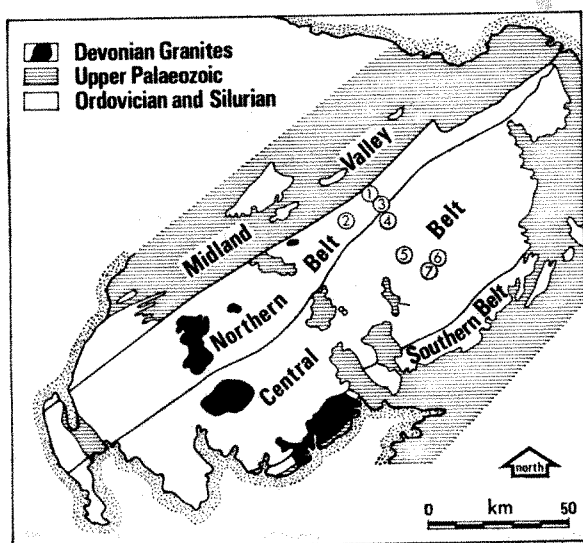


Fig. 1 Map of the Southern Uplands showing the location of the seven sequences in Fig. 2.

Our work in the Northern and Central Belts (unpublished) has substantiated previous observations of the importance of strike faults in the Southern Uplands^{22–28}. Investigation (by J.K.L. and W.S.M.) of sections provided across the strike of the Northern Belt by the recent British Gas pipeline on a traverse to the west of the Clyde Valley has shown that, with only minor exceptions, beds dip at high angles and young predominantly to the north-west. Reverse faults are evident in zones of intense imbrication where the condensed basal basalt-chert-graptolitic mudstone units crop out, but they occur less frequently in the greywacke sections. In the Northern Belt, at least three major reverse faults must be inferred between observed zones of radically different stratigraphy (Fig. 2, sections 1–3). The Central and Southern Belt pipeline sections in Annandale (recorded by M.H.E.) showed steeply-dipping greywackes; though sometimes inverted, they were predominantly

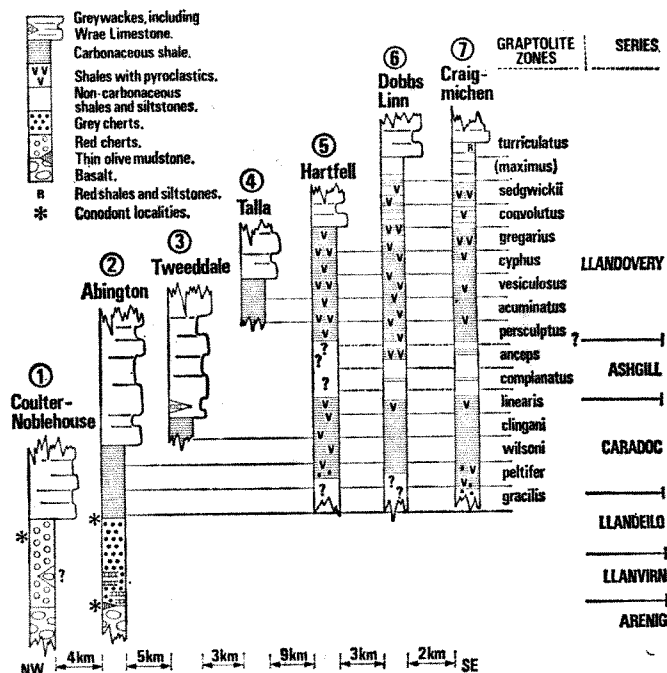


Fig. 2 Ordovician and Lower Silurian stratigraphy in the Southern Uplands: seven sections on a north-west/south-east traverse from the Southern Upland Fault in the Biggar area, Lanarkshire to the Moffat area, Dumfriesshire. The vertical scale reflects the ages of the beds. In the Central Belt (sections 4–7) the total thickness of the graptolitic shales and pyroclastic interbeds is up to about 80 m. Greywacke thicknesses in each fault block may be up to several kilometres. All sections are separated by faults (see Fig. 3). Beds with graptolites are linked by horizontal lines. Conodont localities from Lamont and Lindstrom³¹.

younging to the north-west. Lack of stratigraphic evidence precluded recognition of major fault-bounded sequences from the pipeline alone. A combination of this evidence with the well-established regional stratigraphy^{16,17,27,28}, however, shows significant differences in lithology and age of adjacent sequences (Fig. 2). In particular, it is estimated that the 26 graptolite zones of the Ordovician and Lower Silurian have a total duration of 80 Myr, an average of about 3 Myr per zone. The graptolite faunas thus show that the start of greywacke sedimentation differed by about 3–18 Myr between adjacent sections. We conclude that they were originally deposited far apart, and that they have subsequently been brought together tectonically.

Table 1 Deformation phases in the Moffat Shale of the Central Belt of the Southern Uplands

Style	σ_1	Phase
D ₁	335°	Thrusting southwards at a low angle to bedding, associated cleavage and isoclinal folding
D ₂	320°	Rotation of bedding and thrust planes, monocline formation
D ₃	340°	High-angled strike fault formation
D ₄		Wrench faulting
D ₅		Normal faulting

Structural analysis by M.H.E. from a number of Moffat Shale inliers suggests a complex evolution (Table 1). Initial low-angle southward thrusting, associated with some isoclinal folding was followed by rotation involving steepening, the formation of broad monoclines and steeply dipping strike faults. Such deformational sequences may be seen in certain inliers near Moffat such as at Dobb's Linn and Craigmichen, where beds are thrust over others of the same age but of considerable difference in lithology and thickness. Presumably sheet transportation must have been considerable to account for the juxtaposition of such dissimilar strata.

The model proposed here for the Southern Uplands (Fig. 3) is very similar to those suggested for many modern accretionary prisms. Along the Central American Trench⁶ there is imbricate thrusting and associated folding with progressive steepening, followed by uplift; this shows close analogies with the evolutionary development along such margins as those off Japan⁷, Washington and Oregon⁸, the Makran coast of Iran⁹, Peru¹⁰ and the Aleutians¹¹. Recently uplifted accretionary prism sequences are evident in Barbados^{11,12}, Alaska^{13,29} and the Mentawai Islands (Sumatra)¹⁴. In the Southern Uplands, Cockburnland, situated along what now constitutes much of the Northern Belt, became emergent by Upper Llandovery times and was a source of sediment for both the ocean to the south and the Midland Valley basin to the north²⁸. The younging of successive wedges towards the ocean coupled with overall younging of the sequence within each wedge towards the continent is diagnostic of accretionary prisms; this may be used as a test for their recognition in the geological record.

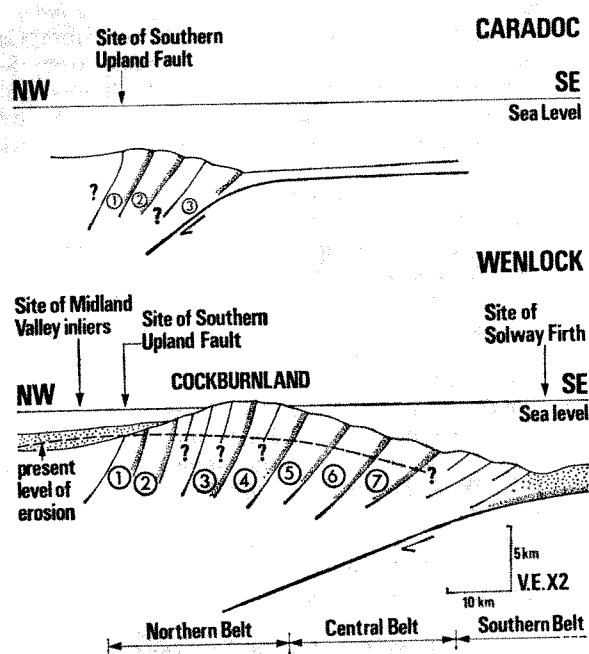


Fig. 3 The Southern Uplands accretionary prism in Caradoc and Wenlock times. Cockburnland was an emergent trench slope break in the Silurian. The numbers 1-7 refer to the sections in Fig. 2. Major faults separate each sequence; in addition, each sequence is affected by many minor faults. Only the known major faults are shown; there may be others, especially in some of the greywacke tracts (for example between sections 2 and 3). The shading at the base of the sections in the Northern Belt represents basalts and cherts, and in the Central Belt it represents shales and occasional cherts. The sediments in the Midland Valley and on the ocean crust to the south are also shaded. The remainder of the rocks are greywackes. The present level of erosion is arcuate to allow for post-Wenlock oversteepening of the prism.

All the evolutionary phases for the accretionary prism are assumed to be diachronous, occurring progressively later to the south. Uplift in the north occurred while accretion was still taking place on the oceanic margin of the Southern Uplands, and while sediments were still being laid down out on the ocean. The oncoming of the greywacke was progressively later to the south (Fig. 2). Similarly, the structural styles are diachronous across the prism; the D₁ to D₃ deformational phases represent only a systematic progression of style as a response to the same tectonic vectors.

With progressive accretion, the ocean margin of the prism migrated about 70 km southwards from a position

close to the Southern Upland Fault in the Llandeilo to the site of the Solway Firth in the Wenlock. Comparison with contemporary examples, such as the Aleutians, suggests that the angle of subduction decreased as the oceanic margin migrated southwards³⁰ (Fig. 3).

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Cretaceous and Tertiary dinoflagellates from Seymour Island, Antarctica

THE marine sediments on Seymour Island, north-eastern Antarctica Peninsula, have produced numerous plant, invertebrate, and vertebrate remains over the past 80 yr (ref. 1). These strata have been regarded as Campanian (late Cretaceous) and Miocene. Dinoflagellate assemblages, reported here for the first time, indicate that some of the Cretaceous beds may be older (Senonian) than previously suspected and that the supposed Miocene beds are instead late Eocene and early Oligocene in age. These Eocene-Oligocene strata are the only known marine sediments of that age exposed on the Antarctic Continent.

The first detailed mapping of Seymour Island began in 1973-1974 (ref. 2). The marine sediments on the island are traditionally divided into two main groups: the Snow Hill Island beds of early and medial Campanian (late Cretaceous) based on ammonites³ and the Seymour Island Series of early Miocene age based in part on fossil penguins^{4,5}. The material examined for dinoflagellates comes from four sections (Fig. 1) measured through these strata^{2,6}. The samples were collected principally by W. A. Bryant (US Geological Survey) and T. A. Trautman (Ohio State University) during the 1974-75 season.

The beds at the north-eastern end of the island adjacent to Cape Wiman that have been mapped as Cretaceous² may be

Palaeocene. A single dinoflagellate assemblage from 63 m above the base near the middle of section S-16 is dominated by an undescribed species and includes *Svalbardella australina*, *Hystrichosphaeridium tubiferum*, *Eisenackia*, and *Palambages*. The presence of these species indicates a Palaeocene correlation, although a possible very late Cretaceous age cannot be entirely discounted.

The northern half of the island, with the exception of the area adjacent to Cape Wiman, contains exposures of the supposed Miocene age Seymour Island Series. The 285-m section from the beach to the west edge of the meseta

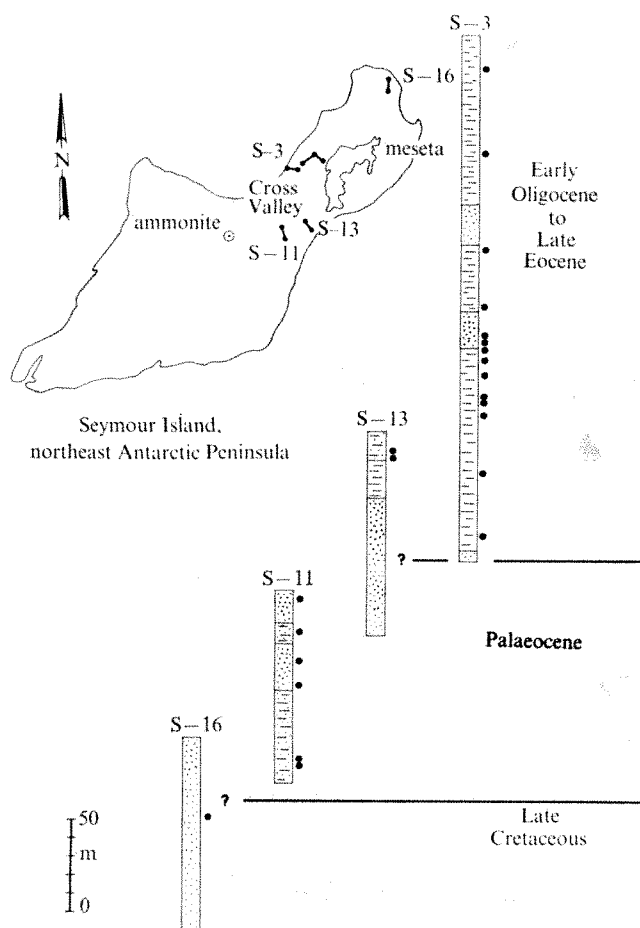


Fig. 1 Seymour Island ($64^{\circ}15'S$, $56^{\circ}45'W$) and generalised stratigraphic sections. Dots to right of columns represent samples from which dinoflagellates were recovered. The maximum length of Seymour is 20.5 km.

(section S-3) includes the oldest beds exposed in that part of the island. Sediments from the lower 84 m of the section contain a diverse dinoflagellate flora that includes *Aerosphaeridium diktyoplokos*, *Deflandrea fuegiensis*, *Deflandrea macmurdoensis*, *Hystrichosphaeridium tubiferum*, and *Spinidinium aperturum*. Above 84 m occur *Aiorea fenestrata*, *Cyclopsiella*, *Thalassiphora*, and *Tubiosphaera filosa*, in addition to the common forms *Deflandrea macmurdoensis* and *Spinidinium aperturum*. These species indicate a late Eocene–early Oligocene age for the Seymour Island Series. The dinoflagellate succession correlates in part with the flora from the Eocene Río Turbio Formation in southern Argentina^{7,8}, and similarities in species composition to the dinoflagellate assemblages from Deep Sea Drilling Project Site 274 of Leg 28 near the Antarctic coast are evident⁹. Several of the above species have been recovered from glacial erratics at McMurdo Sound^{10–13} and from mud

samples grabbed from the bottom of Ross Sea beyond the Ross Ice Shelf¹⁴. Lower Tertiary sediments that are the source of these erratics and dinoflagellates are not exposed in the McMurdo Sound region although beds which correlate with those on Seymour Island undoubtedly exist deeply buried beneath the continental ice sheet. The dinoflagellate evidence supports Simpson's¹⁵ conclusion that the fossil penguin materials from Seymour Island, presumably from the upper part of section S-3 or its equivalent², are no older than late Eocene and no younger than early Oligocene.

Eastern Cross Valley is thought to be the area from which the plant material that was examined for pollen by Cranwell¹⁶ was collected by the Swedish Antarctic Expedition of 1901–1903. The age of the pollen assemblage was initially reported as early Tertiary with recycled late Cretaceous forms¹⁶ and later re-interpreted as Maestrichtian–Palaeocene¹⁷. Section S-13 was measured in the Cross Valley fault zone. Only two samples (130 and 132 m above base) were examined for dinoflagellates. The lower one has a rich spore and pollen content but only one unidentified dinoflagellate; the upper sample is dominated by *Paralecaniella indentata* which in Australia has a stratigraphic range from Campanian to Miocene¹⁸. Neither of the samples from S-13 provides conclusive information. It is likely, however, that the Cross Valley area contains a number of fault slivers which may include both late Cretaceous and early Tertiary sediments.

Section S-11 in the area mapped Cretaceous³ south of Cross Valley may be Palaeocene. Dinoflagellate assemblages from S-11 include *Chlamydomphorella*, *Hystrichosphaeridium tubiferum*, *Palaeoperidinium*, *Spiniferites ramosa ramosa*, *Svalbardella australina*, *Tanyosphaeridium*, *Palambages* spp., and *Paralecaniella indentata*; the ubiquitous *Deflandrea macmurdoensis* and *Spinidinium aperturum* of section S-3 are absent from S-11.

The southern half of Seymour has been dated by ammonite collections as late Campanian¹⁹ and early-medial Campanian³. Matrix from an unidentified ammonite collected by W. J. Zinsmeister (Ohio State University) from that part of the island (Fig. 1) contains the dinoflagellates *Cyclonephelium distinctum* and *Deflandrea cretacea*, indicating an early Senonian age for the ammonite, thus pre-dating the previous ammonite-based Campanian assignment.

Owing to the discovery of several previously undescribed faults in Cross Valley and to the results from the dinoflagellate investigation that differ from other palaeontological studies, final conclusions about the biostratigraphy and correlation of the southern part of Seymour Island will depend on further detailed mapping; at present the extreme southern part of Seymour Island has not been examined.

The abundant well preserved dinoflagellates of Seymour Island provide a new basis for unravelling what seems to be a more complex geology than anticipated. As the dinoflagellate and stratigraphic succession is studied it will become a standard for correlation of the other plant, invertebrate, and vertebrate remains with the better known and more accessible records elsewhere in the Southern Hemisphere.

In addition to abundant dinoflagellates, every sample examined except the one from S-16 contains a very rich assemblage of spores and pollen including several forms of *Nothofagus* and, from S-3, pollen of Gramineae. This is the first fossil record of grasses from Antarctica. Also, massula of the freshwater fern *Azolla* occur in S-11. These late Cretaceous–early Tertiary spores and pollen grains are unstudied but should provide a history of low latitude Antarctica vegetation during the Palaeocene–Oligocene period of climatic cooling before the formation of the present Antarctic Ice Sheet^{20,21}.

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Temporal characteristics of iconic memory

THE duration of visual persistence (iconic memory) is inversely related, up to a point, to the duration of the inducing stimulus. This suggests that iconic memory can more properly be identified with ongoing neural processes than with the decaying contents of a sensory store. Since the publication of Sperling's influential monograph¹ there has been general agreement that a relatively faithful representation of a visual display remains perceptually available for several hundred milliseconds after the distal stimulus has vanished. This led to the postulation of a short-term visual store, also called iconic memory² or sensory register³, with contents decaying rapidly after the termination of the inducing display. The terms visual persistence or icon are commonly used to refer to the perceptual availability of the decaying contents of the sensory store. Iconic memory is regarded as having temporal combinatorial properties which allow perceptual integration of two or more sequential displays even if separated by a brief temporal gap. Integration in iconic memory was convincingly demonstrated in a study where the subjects were required to identify which element in a briefly-displayed array of 16 alphabet characters had been singled out by a temporally trailing bar marker⁴. Temporal integration has also provided an explanation for some aspects of visual masking⁵ and for temporal integration of patterns whose parts were presented sequentially in time^{6,7}. Here I question the view that iconic memory is a store whose contents begin to decay when the inducing stimulus is terminated. I suggest that the duration of iconic persistence is linked to the duration of visual processes which begin at the onset of stimulation and continue for a given duration, whether or not the inducing stimulus is still on display. This view is based on the finding that the duration of visual persistence is negatively related to the duration of the inducing stimulus.

The experimental technique⁷ used a computer-driven oscilloscopic display (gridless Tektronix 602 equipped with ultrafast P15 phosphor) consisting of a square matrix of 25 dots arranged in five rows and columns. One of the 25 dots, chosen randomly on every trial, was not plotted. The subject, who sat in a dark room, initiated each display with a button press and then reported the matrix coordinates of the missing dot. The matrix was displayed in two successive portions separated by a temporal gap: first, an aggregate of

12 dots, chosen randomly from the matrix, was displayed for a period that varied between 10 and 200 ms, depending on the condition; next, a 10-ms temporal gap was allowed to elapse with no dots shown on the screen; and, finally the remaining 12 dots were displayed for 10 ms.

Figure 1 shows the results for two subjects. Almost identical results were obtained with other subjects and with casual observers in the laboratory. A marked impairment in performance is evident as the duration of the leading display exceeded about 100 ms. The phenomenal appearance of the display should be noted: at the shorter durations of the leading portion all dots in the matrix were perceived simultaneously with no evidence of a temporal gap. At the longer durations the display was seen clearly in two successive portions separated by a significant gap.

Since the experimental task was practically impossible unless the two portions of the display were actually seen as one, it seems likely that some sort of visual persistence was necessary for bridging the temporal gap. But could persistence be attributed to the rapidly decaying contents of an iconic store? Probably not. Bearing in mind that the temporal gap was of the same duration in every condition, and that a 'storage' theory assumes an icon whose strength does not start decaying until the termination of stimulation, it is difficult to see how the briefest (10 ms) display could induce iconic contents capable of bridging a given temporal gap while a much longer display could not.

More plausible inferences about visual persistence are, first, that its duration may be more closely timelocked to the onset than to the termination of the inducing stimulus, and second, that it may be more properly considered to be

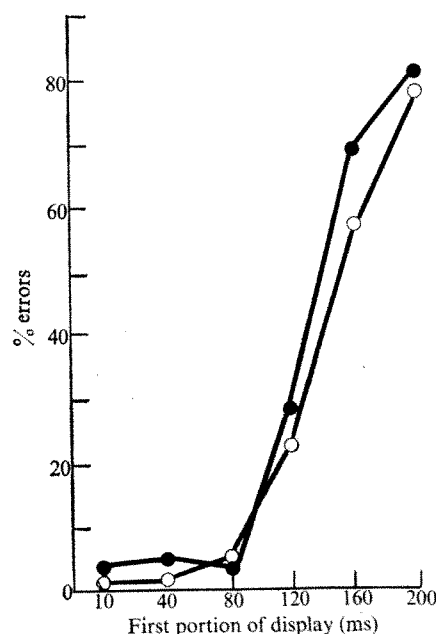


Fig. 1 Percentage of errors in identifying the location of a missing dot within a 5 × 5-element dot matrix which was displayed on an oscilloscope in two portions of 12 dots each, separated by a 10-ms gap. The duration of the first portion of the display is shown on the abscissa; the second portion was always 10 ms. The matrix subtended approximately 6° of visual angle. The two portions of the display were equated for brightness through computer-controlled modulation of the oscilloscope's Z-axis. This allowed independent variation of the intensity of each dot in the display. The appropriate intensity levels for each duration of the leading display had been determined separately in a preliminary psychophysical task. Entirely similar results were obtained without brightness-compensation despite some degree of brightness-mismatch between the two portions of the display. Results are for two subjects—M.K. (●) and V.D.L. (○).

a product of ongoing neural processes of finite duration than the contents of a sensory store.

But before pursuing this line of reasoning, an alternative interpretation must be examined. It may be argued that impairment of performance at the longer durations of the leading display (Fig. 1) may have been due not to reduced visual persistence but to the triggering of some form of discontinuity-detection mechanism within the visual system which effectively segregated the two portions of the display one from the other^{6,8}. To examine this alternative, the display sequence was altered so that each of the 24 dots was displayed once only in random sequence at a regular inter-dot interval (with one exception as noted below). If the inter-dot interval is such that the total duration of plotting of the 24 dots exceeds about 120 ms, the matrix appears to have more than one missing element⁷. The observer is thus confronted with a choice among several apparently empty matrix locations which are indistinguishable from the truly empty location. Over 90% of the errors result from confusing the unplotted dot with dots plotted more than about 120 ms before the termination of the display and hence perceptually unavailable⁷.

The procedure of the second study was a modification of that of Hogben and Di Lollo⁷. The inter-dot interval was fixed at 10 ms corresponding to a total (24-dot) plotting duration of approximately 230 ms. In the control condition each of the 24 dots was plotted once only for 1.5 μ s. In the experimental condition each dot was plotted for 1.5 μ s except for the 12th dot which was plotted for 100 ms (Fig. 2). On the assumption that, up to a limit, the duration of visual persistence is inversely related to the duration of the inducing stimulus, it was expected that the sensory persistence of the 12th dot would be briefer in the experimental than in the control conditions. In turn, it was expected that the 12th dot would be erroneously identified as missing more frequently in the experimental than in the control conditions.

Two observers (the author and a student unaware of the purpose of the research) served in five experimental and five control sessions; each session consisted of 100 trials. The error distributions are shown in Fig. 3, separately for each

Fig. 2 Schematic representation of the plotting sequence. Each of the 24 dots was displayed for 1.5 μ s except for the 12th dot which was plotted for 100 ms. The 12th dot remained on view from the time of plotting of the second dot until 10 ms after the plotting of the 11th dot. Its brightness was indistinguishable from that of all other dots through the compensation procedure described in Fig. 1. The order of plotting was randomised on every trial so that every matrix location had an equal probability of being the 12th in the sequence.

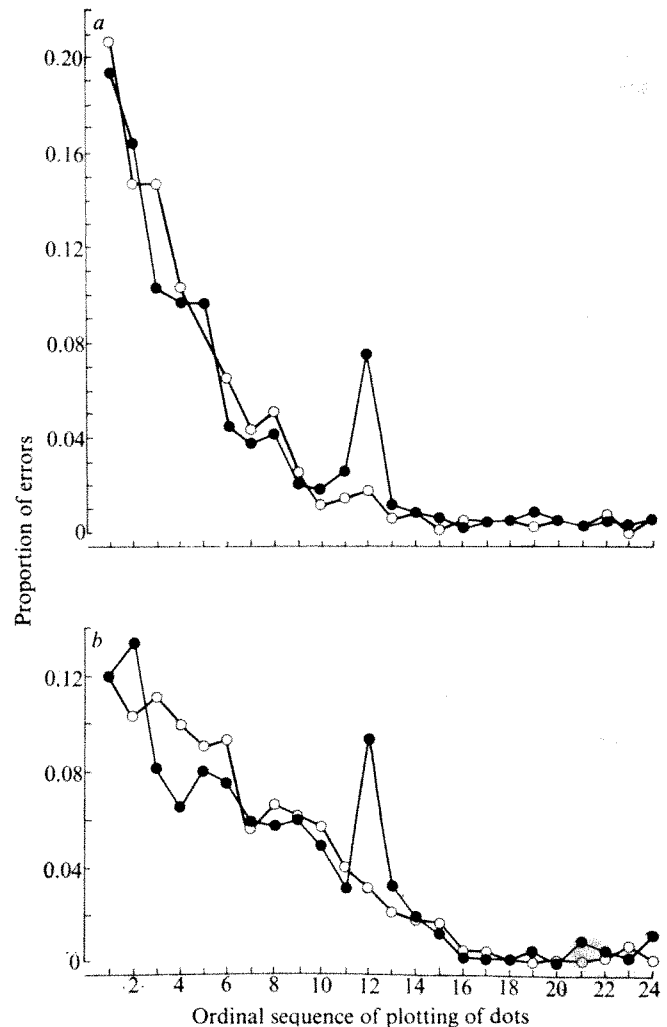
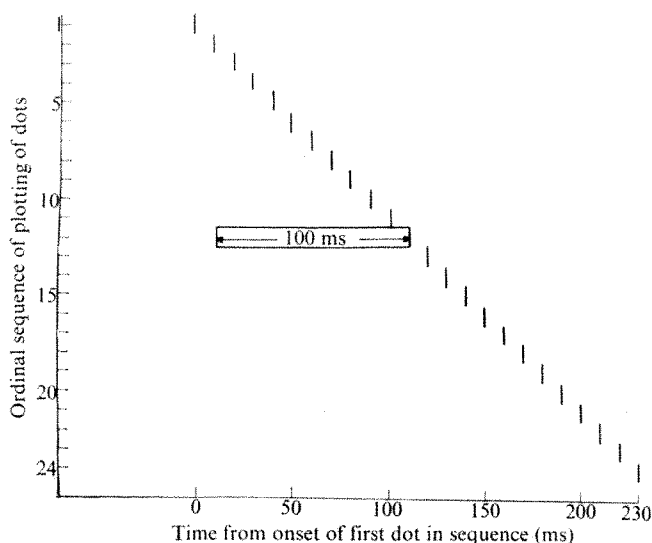


Fig. 3 Temporal distribution of errors in the results of the second experiment. Within each graph, the abscissa indicates the ordinal position of each dot within the plotting sequence, irrespective of spatial location; the ordinate shows the proportion of trials on which a dot plotted in the indicated ordinal position was incorrectly identified as missing. ○, Control condition where every dot was plotted for 1.5 μ s; ●, experimental condition where all dots were plotted for 1.5 μ s except the 12th dot which was plotted for 100 ms. *a*, Subject L.D.C.; *b* V.D.L.

observer. Clearly, the probability of erroneously identifying the 12th dot in the sequence as missing was distinctly higher when its physical duration was long than when it was brief. Similar results were obtained with several other observers.

Unlike the first experiment, where perceptual segregation of the two portions of the display might have been possible, the continuous distribution of the dots over the entire plotting interval made segregation an all but irrelevant consideration in the second experiment. The results also deny the possibility that the termination of the 12th dot might have triggered some sort of discontinuity-detection mechanism within the visual system: had this been so, all dots plotted before the termination of the 12th dot would have become perceptually segregated from the remaining dots. On the contrary, the error distribution (Fig. 3) shows that the dots closely preceding or following the 12th dot in the display sequence were confused with the missing dot much less frequently than the 12th dot.

Contrary to most current thinking^{1-4,9} where iconic memory is said to begin at the termination of a display and to fade rapidly thereafter, the present findings suggest that

iconic memory (as distinct from a retinal after-image) begins at the onset of a display and continues only for a given duration (see also refs 10–13). Thus, iconic memory remains available after the termination of a display only if the duration of the inducing stimulus does not outstrip a given maximum duration from the onset of stimulation. This pattern of results strongly suggests that iconic memory may be more properly viewed as the product of neural processes of finite duration rather than as a sensory store whose contents begin to fade when stimulation terminates. The nature of the processes cannot yet be identified, but there is some support for the view that sensory persistence is produced by the activity of coding mechanisms at the level of feature extraction in visual information processing¹⁴. On this view, persistence would continue while the coding mechanisms are active and would cease at the termination of that processing phase, whether or not the inducing stimulus is still on display.

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Cuckoo among Lake Malawi cichlid fish

THE family Cichlidae consists of bony, perch-like fish which are found in many of the fresh waters of America, Africa and Asia. In Africa most of the almost 700 species occur in the Great Lakes. Approximately 300 species are known in Lake Malawi and 98% of them are endemic to it. Like cichlids from elsewhere, they have intricate but stereotyped territorial and courtship behaviour, followed by spawning and a period of parental care¹. Substrate spawners lay their eggs on the substratum or a suitable object and both parents guard the eggs and later the developing fry^{1,2}. In Lake Malawi the only known substrate spawner is *Tilapia rendalli* Dumeril, a non-endemic species. Evidence suggests that all other cichlids of Lake Malawi are maternal mouthbrooders. When eggs are laid they are picked up by the females and brooded in the mouth^{1,2}. When fry are ready to emerge the parent releases them at a suitable site and usually remains to guard her brood, recalling them into her mouth in the event of danger^{1,2}. After several days, fry no longer respond to their mother's recall signals and the association is terminated. Although cichlid parents usually guard their fry ferociously it seems that at least one species passes these parental duties on to other species of cichlid.

In May 1976, during diving off Likoma and Chisumulu Islands (Lake Malawi), maternal *Haplochromis polystigma* Regan were found caring for mixed broods: their own offspring, easily recognised because they have adult coloration and markings from the outset, and fry of another species differing slightly in size and markedly in coloration. *Haplo-*

chromis macrostoma Regan and *Serranochromis robustus* Regan were also found guarding mixed broods. The foreign fry appeared to belong in all cases to a single species, and differed sufficiently from the native fry to be readily distinguished. When prompted by divers, the guarding parents always retrieved their mixed broods. On occasion, they collected their own fry with greater facility than the foreign individuals, possibly due to a better response of their own progeny to recall signals.

As mixed broods were found on several occasions with each of the three parental species it is unlikely that foreign fry were included by chance. During the 10-d period at Likoma and Chisumulu Islands, *H. polystigma*, *H. macrostoma* and *S. robustus* parents were not found with pure broods.

Members of a mixed brood of *S. robustus* were caught and reared and the cuckoo was identified in January 1977 as *Haplochromis chrysonotus* Boulenger. *H. chrysonotus* is widespread in Lake Malawi and is a member of the Utaka group, which are plankton feeders³.

When the observations were made, shoals of *H. chrysonotus* brooding eggs or fry in their mouths were common in the surface waters. Surrogate parents were found beneath them on the rocks in 2–10 m depth. How the transfer of fry from *H. chrysonotus* to the foster parents takes place is unknown. The three species of foster parent are all active predators and it is conceivable that *H. chrysonotus* forms part of their diet.

It is easy to understand why *H. chrysonotus* progeny should accept foster parents because fry of maternal mouthbrooding cichlids show a very poor parental recognition response. They will follow and attempt to enter crude models which bear no resemblance to the parent in colour, shape, size or markings^{1,4}. Virtually all that is required to induce following is for the model to be moved in a manner which simulates calling behaviour.

It is not easy to understand why foster parents accept and care for fry markedly different from their own. Laboratory experiments^{5,6} with substrate-spawning cichlids have shown that broods could be swapped or mixed provided the progeny were very similar in age, size and behaviour. Dissimilarity of one or more of these criteria invariably resulted in the foster parent eating the foreign fry. Clearly the success of a cichlid cuckoo depends on accurate timing of introductions to the native brood and a similarity of behaviour. Furthermore, provided *H. chrysonotus* fry become intermingled in the foster parents' brood, then even if the parent were reluctant to accept them she would be unable to remove them. A predatory approach towards a mixed brood would evoke an avoidance reaction in all its members. In such circumstances foreign fry would be protected from the surrogate mother by shoaling with her own fry, and protected from other predators by their foster parent when they are taken into her mouth.

Cichlid fry reared with foster parents have been found to prefer the foster parental species to conspecific mates when placed in choice chambers at sexual maturity⁷. In natural conditions, however, *H. chrysonotus* and the three species of foster parent apparently breed true. It is therefore improbable that foster parents become imprinted on *H. chrysonotus* fry to such an extent as to impair subsequent recognition of conspecific rivals and mates.

Although *H. chrysonotus* brood their own eggs in their mouths, they are cuckoos in the sense that foster parents guard their young. This observation raises questions concerning the significance of the parent-brood relationship, the behavioural mechanism(s) of brood hybridisation, and the role of *H. chrysonotus* as a parent.

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Mode of action of insoluble monolayers on mosquito pupal respiration

THE emergence of resistant species and an increased awareness of the environmental problems associated with the use of insecticides has resulted in a search for alternative methods of mosquito control¹. One possibility is lecithin (phosphatidylcholine), which is lethal to the pupae of many species of mosquito and to some fourth stage larvae, when spread as a monomolecular layer at high surface pressure on the air–water interface^{2,3}. Other analogous water-insoluble surfactants, such as cetyl, stearyl and oleyl alcohol ethers of ethylene glycol are equally effective, and successful trials using several of these monolayers have been carried out on rice fields in Kenya⁴. These have not, however, thrown much light on the mechanism of the anoxic process. Observations by high-speed photography suggested that it is not simply a decrease in surface tension, with consequent wetting of the respiratory trumpet of the insect, but also involves a physical barrier to surface penetration (demonstrated in a 35-mm film available from A.I.M.). We report here the importance of the rheological nature of the monolayer, and show that those exhibiting ‘liquid’ properties at high concentrations form efficient barriers while ‘solid’ monolayers do not. We suggest that liquidity at high surface pressure enables (1) rapid repair of the monolayer after penetration or other disturbance; (2) rapid monolayer transfer to the penetrating trumpet during its displacement from the surface followed by ‘wetting’ and the formation of a bilayer-stabilised water ‘plug’, and (3) complete coverage of the water catchment surface in spite of, for example, projecting vegetation.

We applied 0.5 µg of several natural and synthetic lecithins and several semi-synthetic ethoxylated fatty alcohols as solutions in chloroform–methanol (2:1), to clean air–water interfaces within circular dishes of 5 cm internal diameter, each containing 40 pupae of *Aedes (Stegomyia) aegypti* in 8 cm of water at pH 5.8 and 27 °C. This amount of lipid is slightly in excess of that required to give complete coverage as a monolayer. We ensured that the pupae were submerged during the application and evaporation of the solvent. Differential surface

pressures (π) between the monolayer-covered water and clean water (also containing 40 pupae but treated with solvent only) were measured with a null-point electrobalance to each arm of which was attached a mica Wilhelmy plate about 1 cm wide. This gave the monolayer surface pressure as a function of time, with automatic compensation for solvent effects and pupal exudates (if any). The shear rheological properties of the monolayers were determined qualitatively by observation of movements of talc particles sprinkled on the film and gently subjected to an air jet. The surface pressure–molecular area curves obtained in separate experiments on an automated surface balance were used to observe the physical states of the lipid monolayers⁵.

Table 1 summarises the characteristics of the monolayers and their effects on pupae. The gel–liquid crystal transition temperature of lecithin bilayers (T_c) is the critical temperature for the transition from condensed to expanded monolayer⁶. When $T > T_c$, the monolayer is liquid-expanded at all film pressures up to the collapse point. π_s is the maximum film pressure observed when the monolayers were spread from solution and π_e , the equilibrium spreading pressure, is the pressure achieved by spreading from the pure solid⁶.

Table 1 shows that solid, condensed films do not cause death by anoxia, even at surface pressures close to 49 mN m⁻¹ while liquid-expanded films of similar chemical composition are effective at pressures down to about 40 mN m⁻¹. Previous work established a ‘threshold’ effectiveness at approximately 45 mN m⁻¹ (ref. 3). A simple explanation of the difference between solid and liquid films is that the solid film can readily be broken and partially collapsed by pupae; the hole is not immediately repaired because the lecithin cannot respread spontaneously because the value of π_e is zero^{5,6} and the solid ethoxylate film spreads to only 34 mN m⁻¹. On the other hand, similar breaks in liquid-expanded films are repaired quickly (because $\pi_e \approx 50$ mN m⁻¹), the rate depending on surface viscosity. Thus in the latter case, pupae are always faced with a complete and continuous monolayer at a very high surface pressure; this very high lateral pressure will increase the work required to create a hole in the film and tend to squeeze any trumpets out of the interface. We now consider the mechanism by which such films lead to anoxia of pupae.

In normal conditions, the pupal trumpet breaks surface readily without movement of the pupa other than vertical buoyant rise. Even in the presence of a liquid monolayer at $\pi > 45$ mN m⁻¹, pupae can usually gain access to the atmosphere at the first attempt but in subsequent attempts they struggle vigorously against the film, often concentrating the effort on one trumpet by attacking the surface sideways. When penetration is prevented, the air sac within the trumpet can be seen clearly like a stabilised foam bubble which does not coalesce with the atmosphere. Thus the interior of the trumpet can be represented as a bubble of air enclosed within a hydrophobic layer of material rising towards the surface (Fig. 1A). As the trumpet reaches the surface, water molecules must drain from between the two air phases, a^* and a , for these to coalesce, giving direct access of the respiratory system

Table 1 Characteristics of monolayers

Lecithin (or analogue)	Chain length and degree of unsaturation	T_c (°C)	Monolayer state at 27 °C	π_s (mN m ⁻¹)	π_e (mN m ⁻¹ at 27 °C)	% Pupae dead after 12 h at 27 °C
Egg	Mainly					
Total egg lipids	C ₁₆ ^{18:1}	–15 to –5	Liquid-expanded	50	50	100
Di-oleoyl	—	—	Liquid-expanded	48	48	100
Didecanoyl	C _{18:1}	–22	Liquid-expanded	46	46	100
Dimyristoyl	C ₁₀	< 0	Liquid-expanded	45	—	100
Dipalmitoyl	C ₁₄	23	Liquid-expanded	52	50	100
Distearoyl	C ₁₆	41	Solid, condensed	49	0	0
Dibehenoyl	C ₁₈	58	Solid, condensed	49	0	0
Oleyl monoethoxylate	C ₂₂	75	Solid, condensed	37	0	0
Cetyl/stearyl monoethoxylate	C _{18:1}	—	Liquid expanded	41	39	100
	C ₁₆ ¹⁸ /C ₁₈ ¹⁸	—	Solid, condensed	46	34	0

to the atmosphere. When the air-water interface in contact with phase a^* and the trumpet surface is devoid of surfactant molecules, drainage is rapid and rupture of the thin water film is easy (Fig. 1B); the hydrophobic trumpet is dewetted quickly because the receding contact angle θ_R of clean water is finite. This situation holds whenever the pupa penetrates a monolayer at less than the threshold surface pressure or for the first penetration of a monolayer at $\pi > 45 \text{ mN m}^{-1}$.

If a pupa detaches from a lecithin monolayer at $\pi > 45 \text{ mN m}^{-1}$ the bubble a^* becomes covered with a monolayer at high π and on subsequent contact with the surface the trumpet does not dewet quickly, because the air-water θ_R is zero (Fig. 1C and D). Brooks *et al.*⁷ have shown that θ_R for an aqueous surface covered with dipalmitoyl lecithin on low energy solids such as siliconed glass decreases to zero when $\pi \approx 45 \text{ mN m}^{-1}$ (and tends towards zero when $\pi \approx 40 \text{ mN m}^{-1}$ in the case of octadecanol which is non-ionic and analogous to the ethoxylates); this wetting involves deposition of lecithin at the solid-vapour or solid-liquid interface. Because normal dewetting is thereby eliminated, a thin water film is maintained across the mouth of the trumpet and an air channel cannot be formed easily. In this situation, the thin film of water is analogous to that between air bubbles in a foam and it persists because the highly compressed lipid monolayers retard drainage of water from the film; ultimately a black film could be stabilised by interactions between the two monolayers⁸.

Although some species may penetrate a monolayer several times, progressive deposition of lipid and wetting will occur within the trumpet and it will become more and more difficult for the thickening plug of water (Fig. 1D) to drain in a reasonable time. Electron micrograph studies with *Culex pipiens fatigans* suggest that in such instances layers of surfactants are built up on the 'pedepodial' filaments within the trumpet, presumably destroying their function of resisting water invasion. That such deposits (Fig. 2) may be built-up multilayers of lipid is suggested by the observation that lipid multilayers can be deposited on a low energy surface such as PTFE (ref. 9).

Fig. 1 Normal dewetting of the hydrophobic pupal trumpet. In the absence of surfactant the thin film of water across the mouth of the trumpet is unstable because of the high free energies of the two surfaces. After rupture, the clean water recedes from the trumpet because the receding contact angle is finite (A and B). The effects of an insoluble monolayer with equilibrium spreading pressure $\pi_e > 45 \text{ mN m}^{-1}$ on the interaction of the pupal trumpet with the water surface are also shown. On the first approach (A) and (B) still apply because the air-water interface in contact with air inside the trumpet (a^*) is clean and the trumpet penetrates successfully. On subsequent approaches (C and D) this interface is covered with a monolayer of lecithin so that spontaneous dewetting of the trumpet no longer occurs, the receding angle now tending to zero, while the hydrated bilayer film is stabilised by minimal free energy interfaces.

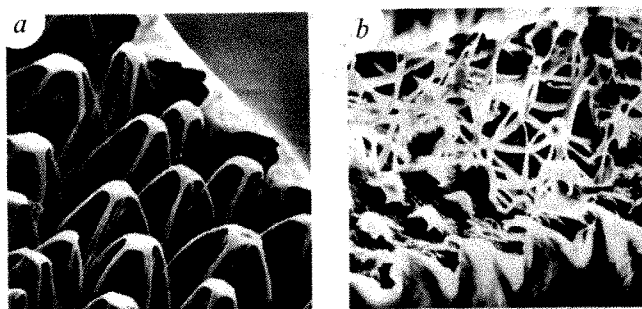
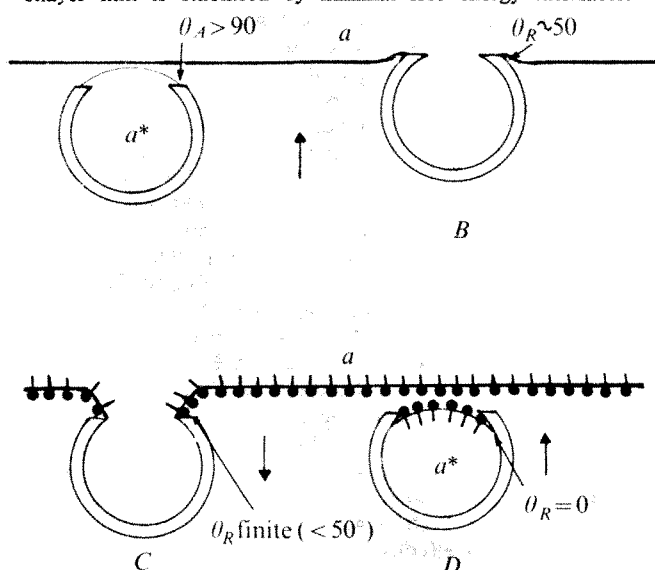


Fig. 2 *a*, An electron micrograph of the 'pedepodia' structures within a normal *Culex p. fatigans* pupal trumpet. *b*, A similar view of a specimen killed by lipid monolayer anoxia.

Laboratory and field studies indicate that many aquatic organisms ('non-target' in the context of mosquito control) are not killed by monolayers and we believe this to be due to the rather specific mechanism which we describe. Even the larvae of mosquito are not killed directly by monolayers, but are inhibited from surfacing until the dissolved oxygen content of the catchment falls below a critical value overnight¹⁰ when they are anoxiated.

When spread on tropical rice fields at about ten times the amount required for monolayer coverage, natural lipids and the semi-synthetic ethoxylates maintain their full surface pressure for about 1 and 3 d respectively. In both cases the monolayers, with their associated 'lenses' of reservoir material, effectively remove mosquitoes for periods of more than 10 d, after which further application would be required to give complete control.

These observations and our suggested mode of action indicate that natural lipid or other high-pressure monomolecular films afford a method of mosquito control which is more selective, more efficient and more ecologically acceptable than methods involving toxic chemicals. In addition, the physical nature of the mode of action which we have outlined may be less likely to encourage the selection of resistant mutants.

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Glutamine synthetase and control of nitrogen fixation in *Rhizobium*

Rhizobium, a bacterium which reduces dinitrogen to ammonia in symbiotic association with leguminous plants, 'fixes' nitrogen while living free¹⁻⁴. Unfortunately, free-living fixation seems to occur only at the end of exponential growth, and no condition is known in which bacterial growth is dependent on reduction of dinitrogen by nitro-

genase (EC 1.7.99.2). Therefore a molecular biological analysis must be indirect. In the unrelated *Klebsiella pneumoniae*, where fixation does occur in culture, nitrogenase expression is regulated by the enzyme glutamine synthetase (GS, EC 6.3.1.2). By analogy, we have isolated GS auxotrophs in *Rhizobium* and screened for nitrogenase activity. We chose *Rhizobium* cowpea 32H1 because it has the highest reported nitrogenase activity, both in culture and in nodules⁵. We found that a glutamine-dependent auxotroph of *Rhizobium* cowpea 32H1, deficient in GS activity, is deficient in nitrogenase activity as well. Therefore GS seems to be involved in the control of nitrogenase expression by *Rhizobium*.

In *K. pneumoniae*, mutants lacking GS activity (*gln*⁻) fail to induce measurable nitrogenase activity^{6,7}. Moreover, constitutive mutants (*Gln*^o phenotype), in which GS activity is no longer repressed by ammonia, are also constitutive for nitrogenase activity. On these grounds GS has been proposed as a positive regulator of nitrogenase.

In enteric bacteria GS has both catalytic and regulatory functions. Different GS assay procedures may yield different results. One assay (the 'transferase' reaction) measures ADP-dependent γ -glutamyl hydroxamate formation⁸. In this reaction the adenylylated form is still partially active. The adenylylated and deadenylylated forms of the enzyme GS, however, respond differently to changes in pH and Mg²⁺ concentration. Therefore quantification of GS levels is difficult unless the state of adenylation of GS is known⁹.

We have used the 'transferase' assay for rhizobial GS. Our finding that a GS auxotroph is deficient in nitrogenase is consistent with a similar regulatory role for GS in *Rhizobium*. We wish, however, to emphasise that little is known about rhizobial GS with regard to parameters such as adenylation and assimilation of ammonia at high and low concentrations.

GS transferase activity in wild type 32H1⁺ is shown in Table 1. Activity was five times higher in M + glutamate than in either M + glutamine or M + ammonia. This

Table 1 Glutamine synthetase activities of *Rhizobium* 32H1 strains

Strain	Growth medium	GS transferase activity (U per 10 ⁷ cells)
32H1 ⁺	M+glutamate	2.0
	M+glutamine	0.41
32H1 <i>gln5</i>	M+glutamate	—
	M+glutamine	0.13
32H1 <i>gln5r</i> 101 (<i>Gln</i> ⁺)	M+glutamate	0.47
	M+glutamine	0.39

To isolate glutamine auxotrophs, cells of 32H1⁺ were grown to 5×10^8 per ml in liquid cultures of YAP medium (0.1% yeast extract, 0.1% arabinose, 7.5mM phosphate, pH 6.3). Cells were incubated in citrate buffer, pH 5.5 containing *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (0.1 mg ml⁻¹) for 1 h at 30 °C and then washed with phosphate buffer, pH 6.3. Mutagenised cells were grown overnight in YAP and then resuspended in a minimal defined medium (M) containing: 0.1% arabinose, 0.8 mM MgSO₄, 0.05 mM CaCl₂, ferric citrate (10 μ g ml⁻¹) and 7.5 mM potassium phosphate, pH 6.3. Glutamate (0.1%) was added as nitrogen source and the culture was incubated at 30 °C for 24 h to deplete amino acid pools. At this time penicillin G (5 mg ml⁻¹) and lysozyme (0.1 mg ml⁻¹) were added and shaking was continued for a further 24 h. Surviving cells (approximately 0.1%) were treated with penicillinase and plated on YAP plus glutamine (0.1%). Among 1,500 colonies tested, three grew only when supplemented with glutamine. The reported experiments were conducted with one of these, *gln5*. GS was assayed by the glutamyl transferase reaction described in the text. 32H1⁺, 32H1 *gln5* or independently isolated revertants growing exponentially at 30 °C in M+glutamate (0.1%) or M+glutamine (0.1%) were collected by addition of cetyltrimethylammonium bromide (0.1 mg ml⁻¹) and centrifuged in the cold. Cell pellets were washed once with 1% KCl, centrifuged and resuspended tenfold concentrated in the same. Assays were performed as before⁹, except that the pH was 7.00, where rhizobial GS transferase activity was a maximum. Twenty-five *Gln*⁺ revertants of *gln5* assayed yielded the same non-derepressible phenotype when grown on M+glutamate as is shown by revertant 101.

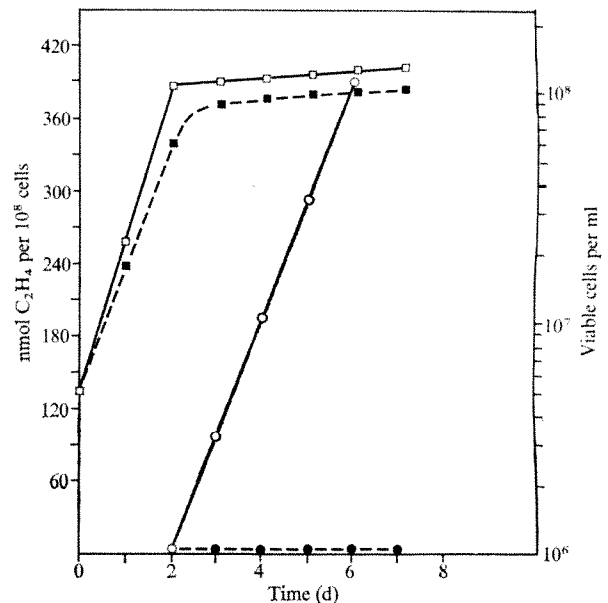


Fig. 1 Nitrogenase induction in liquid culture of 32H1 compared with 32H1 *gln5*. Nitrogenase was assayed by acetylene reduction in liquid culture¹⁰. 32H1⁺ and 32H1 *gln5* were grown in YAP, centrifuged, washed with phosphate buffer twice and resuspended in M+glutamate (0.1%), glutamine (0.002%) and NaMoO₄ (10 μ g ml⁻¹). Cultures (3 ml) were injected into 30-ml capped serum bottles; the bottles were evacuated to 0.1 atm and the atmosphere was replaced with 95% argon-5% CO₂ four times in succession. O₂ (0.001 atm) and C₂H₂ (0.1 atm) were injected and cultures were rotated vigorously at 30 °C. Aliquots were withdrawn and plated on YAP + glutamine (0.1%) for cell counts: □, 32H1⁺; ■, 32H1 *gln5*; or assayed gas chromatographically for ethylene production: ○, 32H1⁺; ●, 32H1 *gln5*.

suggests that GS is sensitive to repression by ammonia in *Rhizobium*, as in *Klebsiella*; repression in M + glutamine might result from ammonia formed from hydrolysis of glutamine. (We will present a more complete study of repression by ammonia elsewhere.)

GS levels in the mutant *gln5* can be measured only in repressing conditions (M + glutamine). Table 1 shows that *gln5* had little or no GS activity, although a residual γ -glutamyl hydroxamate formed in the 'transferase' assay.

We cannot tell whether the lesion of *gln5* is in the structural gene for GS or elsewhere. For what follows, the important point is that GS activity in *gln5* is negligible.

Figure 1 shows nitrogenase activity in cultures of 32H1⁺ and 32H1 *gln5*. The mutant had no nitrogenase activity above background. Nitrogenase activity was assayed in liquid cultures¹⁰ by the reduction of acetylene to ethylene^{11,12}. Cells were inoculated at 10⁶ per ml. Before detection of nitrogenase activity colony counts reached 10⁸ per ml and then remained essentially unchanged for up to 10 d regardless of whether acetylene was present for nitrogenase quantification. If the vials were opened to the air, exponential growth resumed after some lag and continued to at least 5×10^8 cells per ml, showing that growth factors, particularly nitrogen, had not been depleted. Glutamine was present at an unusually low level (0.002%) because higher levels inhibit nitrogenase induction in 32H1⁺.

To study the effect of the *gln5* allele on symbiotic nitrogen fixation, 32H1⁺, 32H1 *gln5* and *Gln*⁺ revertants of *gln5* were allowed to nodulate the tropical legume *Macropituleum atropurpureum*. Seedlings nodulated with 32H1⁺ showed high levels of nitrogenase activity whereas there was no detectable activity either in plants nodulated with *gln5* or in uninoculated, un-nodulated plants (Fig. 2). *gln5*-inoculated seedlings grown for a further 14 d as a precaution against slow maturation still had no nitrogenase activity.

Seedlings with effective nodules were green and healthy, whereas ineffectively nodulated seedlings began to yellow

Table 2 Nitrogenase activities and phenotypes of viable *Rhizobium* of *M. atropurpureum* nodules

Strain inoculum	Viable cells of <i>Rhizobium</i> * per nodule	Phenotypes*		Nitrogenase activity†
		Gln ⁺ (%)	Gln ⁻ (%)	
32H1 ⁺	7.0 × 10 ⁶	100	—	204
32H1 <i>gln5</i>	5.8 × 10 ⁶	83	17	<1
r101 (Gln ⁺)	6.1 × 10 ⁶	100	—	112
r102	3.6 × 10 ⁶	100	—	<1
r111	8.0 × 10 ⁶	100	—	<1
r113	3.2 × 10 ⁶	100	—	<1
r114	4.1 × 10 ⁶	100	—	86
r16L	5.3 × 10 ⁶	100	—	<1

Seedlings were grown and nodulated with the given strains of *Rhizobium* 32H1⁺, 32H1 *gln5*, and Gln⁺ revertants as described in Fig. 2. Assays for nitrogenase were conducted as described in Fig. 2. Nodules were excised, immersed momentarily in ethanol, then in 3% H₂O₂ for 5 min, crushed in 1.0 ml of phosphate buffer, pH 6.3 and plated on YAP and YAP + glutamine (0.1%) for viable cells of *Rhizobium*.

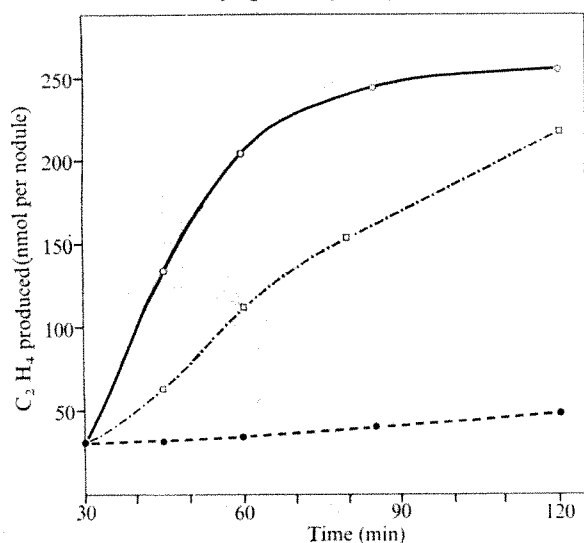
*Mean number of six nodules.

†Net activity in nmol of ethylene produced per h per nodule minus that produced by uninoculated, un-nodulated seedlings.

due to acute nitrogen starvation. Both ineffective and effective nodules were then crushed and plated for counts of viable *Rhizobium* with both Gln⁻ and Gln⁺ phenotypes. We were surprised that nodules resulting from *gln5* inoculation yielded viable *Rhizobium* in a mixture of both phenotypes, with Gln⁺ predominating (Table 2).

Nodulation with both 32H1⁺ and 32H1 *gln5* was repeated with various amounts of glutamine (0.01–1.0%) added to the seedling media. No nodules were seen on plants inoculated with either strain in the presence of added glutamine. But between one and five nodules per plant eventually developed during the next 14–28 d. As expected, nodules produced from *gln5* inocula had no nitrogenase activity. Again, aseptic crushing and plating for viable *Rhizobium* revealed populations with mixed Gln⁻ and revertant Gln⁺ phenotypes. Therefore *gln5* might itself nodulate and the Gln⁺ colonies represent simply accumulated revertants. Alternatively, *gln5* might be deficient at some stage of nodulation at which reversion to prototrophy is required. In this case *gln5* would only passively inhabit nodules formed by revertants.

Fig. 2 Nitrogenase activity of *M. atropurpureum* nodules. Seeds were surface sterilised in hypochlorite solution (2.5%) for 30 min, sprouted and placed in 70 × 25-mm tubes with filter paper supports. Seedlings were grown in nitrogen-free liquid medium¹⁷ inoculated with 10⁶ exponentially growing 32H1⁺ (○), 32H1 *gln5* (●) or 32H1 Gln⁺ revertant r101 (□) 7d after germination. After 28 d of growth at 29 °C with a constant 14-h photoperiod, well-nodulated seedlings were capped and injected with C₂H₂ (0.1 atm). Aliquots were withdrawn periodically and assayed for ethylene production by gas chromatography^{11,12}. No ethylene production was observed in uninoculated, un-nodulated seedlings or *gln5*-nodulated seedlings. Activities are given per effective nodule, which was determined by subsequently excising individual nodules and assaying for ethylene production.



In culture, 32H1 *gln5* reverts to prototrophy at a rather high frequency of 1×10^{-5} per cell per generation. Six such independently isolated Gln⁺ revertants of *gln5* were tested for nodulation and activity (Table 2). Two gave nodules that reduced acetylene at rates similar to 32H1⁺ nodules. The remaining four gave nodules that looked healthy but had no detectable nitrogenase activity. This suggests that there are two classes of Gln⁺ revertants: those that regain both catalytic and regulatory activities, and those that regain catalytic activity only. (We have no selection for revertants that regain regulatory activity only.)

These results indicate that GS plays a role in the regulation of nitrogenase activity in both free-living *Rhizobium* and bacteroids, although the mechanism is still unclear. Kondorosi *et al.*¹³ have isolated a glutamine-requiring derivative of the 'fast-growing' species *R. meliloti* 41 his⁻ from among the revertants able to grow on D-histidinol. This glutamine auxotroph yields nodules on *Medicago sativa* which do not have nitrogenase activity, a finding similar to our own. The results of Brown and Dilworth¹⁴ with bacteroid preparations suggest that glutamine synthetase–glutamate synthase-directed ammonia assimilation does not occur in the bacteroid, but rather in the associated plant cell where the same two plant enzymes are present at high levels. These plant extracts also had NAD- or NADP-linked glutamate dehydrogenase activities. Bergerson and Turner¹⁵ showed that all ¹⁵N₂ reduced by *R. japonicum* bacteroids appeared rapidly as ¹⁵NH₄⁺ in supernatant fractions, supporting the involvement of plant ammonium assimilatory enzymes in fixation. O'Gara and Shanmugam confirmed that 94% of the ammonium ion produced by nitrogen fixation in free-living *R. japonicum* is exported as ammonium ion¹⁶.

In both free-living and bacteroid states, rhizobial GS seems to be present during fixation at low levels, insufficient to utilise all the ammonium ion produced by nitrogenase. Because bacteroids are inviable differentiated forms, rhizobial GS might be involved in shutting off the assimilation of bacteroid ammonia as part of the overall shift in nodule metabolism from bacterial to plant cells.

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Nitroso group exchange as a way of activation of nitrosamines by bacteria

MUTAGENIC compounds and most carcinogens are thought to interact either directly or through metabolic activation processes with DNA to alter and damage the double helical structure. Bacteria defective in some aspect of DNA repair or recombination are in general much more sensitive to mutagenic and carcinogenic agents than the parent strains. Several bacterial test systems^{1,2} have been developed to assay for carcinogenic activity based on the premise that most carcinogens are mutagens in the proper conditions. One of the most widely used bacterial screening systems has been developed by Ames *et al.*¹ based on mutational reversion to prototrophy of a sensitive strain of *Salmonella typhimurium*. We have developed a screening system using *rec⁻ Escherichia coli* based on the lethal nature of unrepaired DNA³⁻⁵.

During the development of this screening system we discovered that our test bacteria, a *recB⁻ recC⁻* (*recBC*) strain obtained from A. J. Clark, Berkeley, California, was very sensitive to a mixture of 2-acetyl aminofluorene (AAF) and dimethylnitrosamine (DMN), neither of which alone

Fig. 1 Alkaline sucrose gradient analysis of *E. coli* AB1157. Cultures in exponential growth were labelled with ³H-thymidine, 3 µCi ml⁻¹ (specific activity 50 Ci mmol⁻¹) at 37 °C for 1 h in the presence of 2-deoxyadenosine, 250 µg ml⁻¹. Bacteria were then centrifuged, resuspended in 0.1 M phosphate buffer (pH 7.0) and treated with DMN and/or AAF for 20 min. They were then washed twice to remove the carcinogens and resuspended in 0.1 M phosphate buffer (pH 7.0). Following this, 50 µl (~10⁸ cells) were layered gently on 0.15 ml lysing solution (0.15 N NaOH, 0.40 M NaCl, 0.01 M Na-EDTA and 0.3% sarcosyl) placed on top of 4.3 ml alkaline sucrose gradient (5-20% sucrose, 0.9 N NaOH, 0.1 M NaCl and 0.001 M Na-EDTA) and a shelf of 0.5 ml 70% sucrose. The gradients were allowed to stand for 1.5 h or 90 min at room temperature to ensure complete lysis of cells and then centrifuged for 1 h at 40,000 r.p.m. in a SW65 rotor in a Beckman L-65 ultracentrifuge. Eight drop fractions were collected from each gradient and sample counted in a Triton 100-toluene fluor. ●, AAF 25 µg ml⁻¹; □, DMN 2.5 × 10⁻¹ µg ml⁻¹; ○, AAF 25 µg ml⁻¹ plus DMN 2.5 × 10⁻¹ µg ml⁻¹.

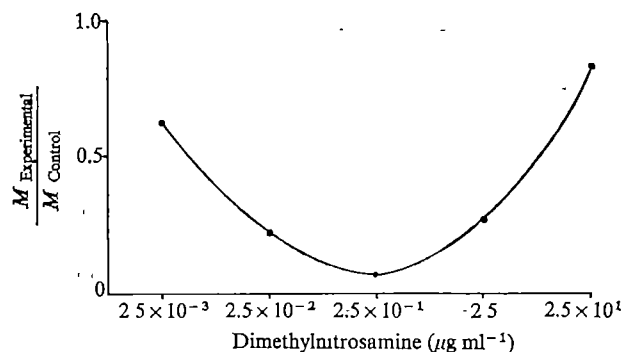
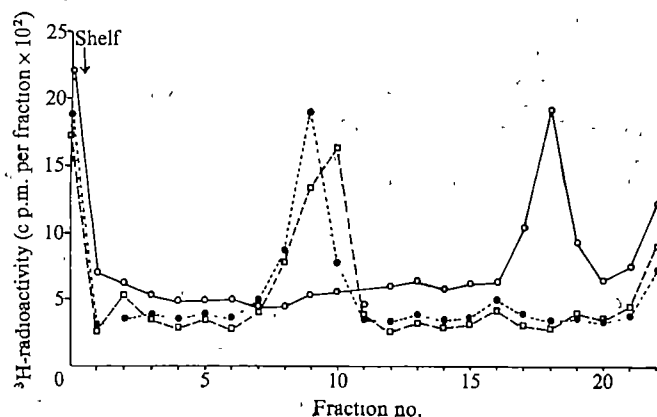


Fig. 2 Alkali sensitivity of bacterial DNA as a result of exposure of bacteria to AAF plus DMN. Using the procedures given in Fig. 1, a number of alkaline sucrose gradients were analysed in which the AAF concentration was kept constant at 25 µg ml⁻¹ and the DMN concentration was varied from 2.5 × 10⁻³ to 2.5 × 10⁴ µg ml⁻¹. The ratio of the molecular weight of the DNA of bacteria treated with AAF plus DMN (*M* experimental), to the molecular weight of the untreated control (*M* control) or to the molecular weight of the bacteria treated with AAF or DMN alone was obtained from the relationship:

$$\frac{\text{Distance}_1}{\text{Distance}_2} = \left\{ \frac{M_1}{M_2} \right\}^{0.4}$$

as developed by Studier⁶.

had any effect on the growth of JC5519 (*recBC*). In the presence of 25 µg AAF, the assay could detect as little as 2 × 10⁻² µg DMN without the requirement for metabolic activation which is normally required for DMN. Was this synergistic effect acting in a 'classical' manner on the genome of the bacteria?

Cells of strain AB1157 (the parental strain isogenic with JC5519), whose DNA had been radioactively labelled, were exposed to AAF, DMN and a mixture of AAF and DMN, then lysed on top of an alkaline sucrose gradient and sedimented⁶. Figure 1 presents the results of such gradients which show quite clearly that the combination of AAF and DMN produce alkali-labile lesions (single-strand breaks) in DNA. On the other hand, AAF and DMN alone have no effect on the DNA of the bacteria as observed in alkaline gradients with control bacteria. If the AAF concentration is kept constant and one varies the DMN concentration, the fragment size as observed in alkaline sucrose gradients first decreases as the DMN concentration increases, reaches a minimum size and then increases as shown in Fig 2. At high levels of DMN the combination of AAF and DMN has no effect on the bacterial DNA probably due to cytotoxic effects.

To explore the hypothesis that a bacterial enzyme was involved in this interaction between AAF and DMN, we incubated bacterial extracts from both AB1157 (parent strain) and JC5519 (*recBC*) with AAF and DMN, and tested them with the Ames tester systems. AAF and DMN without microsomal activation have a negligible effect in producing revertants in the *his⁻ S. typhimurium* tester strain; however, in the presence of sonicated bacterial extracts from AB1157 or JC5519, the combination of AAF and DMN became mutagenic in the standard Ames assay (Table 1). As a next step, after incubation of AAF and DMN with a sonicated cell-free bacterial preparation, we extracted the reaction mixture with methylene chloride, evaporated the methylene chloride fraction to dryness, and dissolved the residue in DMSO. This DMSO extract was also active in producing revertants in *S. typhimurium*. These results are presented in Table 1. In Fig. 3 we have plotted the results from a number of assays with the Ames tester strain; the AAF concentration was kept constant and the DMN concentration varied in the presence of JC5519 bacterial extract. The number of revertants rise with DMN

Table 1 Mutagenic activity of cell-free preparations of *E. coli*, AAF and DMN, using *S. typhimurium* TA98

Compounds of mixture	Mutation ratio TA98
1 AAF+DMN+JC5519 prep	2.8
2 AAF+DMN	1.0
3 DMN+JC5519 prep	1.0
4 AAF+JC5519 prep	1.0
5 AAF+DMN+JC5519 prep (heated)*	1.0
6 AAF+DMN+JC5519 Prep (pronase treatment)†	1.0
7 AAF+DMN+AB1157 prep	1.7
8 AAF+DMN+CH ₂ Cl ₂ extract‡ of JC5519	2.3
9 AAF+benzylphenyl nitrosamine+JC5519 prep	2.5
10 AAF+nitroso-pyrrolidine+JC5519 prep	2.4

E. coli JC5519 were grown overnight on nutrient broth, and collected by centrifugation at 4,000g for 15 min. The bacteria were resuspended and washed twice in phosphate buffer (0.1 M, pH 7.0) and finally suspended in 50 ml to give about 10⁹ cells ml⁻¹. The cell suspension was sonicated at full power in a Bionsonik sonicator for 5 min, and cooled in an ice bath. The sonicated preparation was centrifuged three times at 4,000g for 15 min to remove intact bacteria. The final supernatant was used for subsequent experiments. The protein concentration of the preparation was routinely 2.0 mg ml⁻¹ by Folin-Lowry.

To measure the number of *Salmonella* revertants, 1.0 ml of the sonicated *E. coli* preparation, 2.5 µg DMN and 25 µg AAF were incubated for 15 min at 37 °C. The reaction mixture is then mixed in 3.0 ml 0.75% minimal histidine soft-top agar and 10⁶ cells of *S. typhimurium* TA98 of Ames¹ are added. The mixture is poured over minimal agar plates and incubated at 37 °C for 48 h. The mutation ratio is the number of colonies on the experimental plate to the number of colonies on a control plate which is inoculated with the same number of tester bacteria.

*Cell-free extract heated for 15 min in boiling water bath before incubation with DMN and AAF.

†One ml of cell-free extract pre-incubated with 0.1 mg Pronase 20 min before addition of DMN and AAF.

‡DMN (2.5 µg), AAF (25 µg) and 1 ml cell-free extract of *E. coli* were incubated for 15 min. CH₂Cl₂ (5 ml) was added, the mixture shaken, the phases separated after standing 10 min and methylene chloride phase-evaporated to dryness under a stream of N₂; the residue was dissolved in 1.0 ml DMSO and added to 3.0 ml soft-top agar with 10⁶ cells of TA98 and poured over minimal agar plate in a standard Ames test.

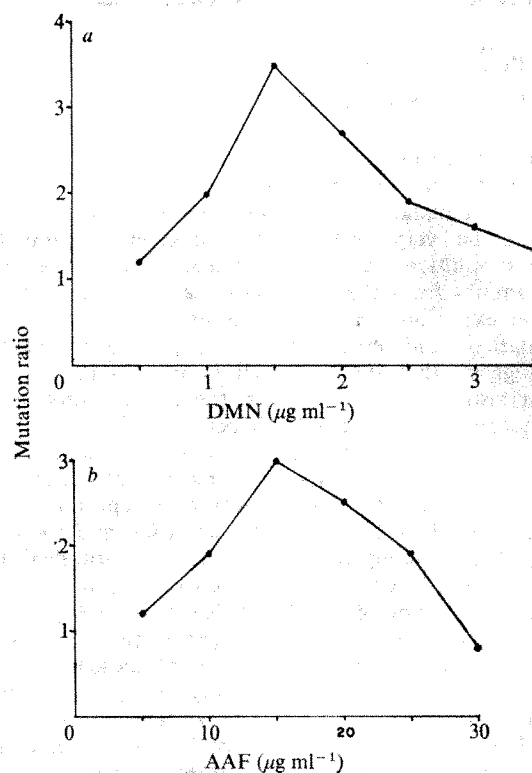
concentration and then decrease with higher concentration of DMN (Fig. 3a). A similar curve was obtained by varying the AAF concentration at constant DMN concentration (Fig. 3b).

Nitrosamides are known not to require activation⁷ by liver microsomal enzymes and are capable of reacting directly with DNA. It is possible that these observations reported here (Figs 1 and 2; Table 1) are the result of a nitroso group exchange reaction in which the nitroso group is transferred from the nitrosamines to the amide moiety of AAF. To gain support for this hypothesis an experiment (Table 2) was carried out in which AAF was replaced with a

Table 2 Mutagenic activity of a cell-free preparation of *E. coli*, DMN, various amides and other substances, using *S. typhimurium* TA98

Compounds of mixture	Mutation ratio TA98
1 AAF+DMN+JC5519 prep	2.8
2 Urea+DMN+JC5519 prep	2.6
3 Urea+JC5519 prep	1.0
4 DL-alanyl-DL-alanine+DMN+JC5519 prep	1.9
5 Glycyl-L-tyrosine+DMN+JC5519 prep	2.3
6 Choractyl-L-tyrosine+DMN+JC5519 prep	2.2
7 DL-alanyl glycine+DMN+JC5519 prep	1.9
8 L-phenylalanyl-L-phenylalanine+DMN+JC5519 prep	2.5
9 N-acetyl-L-tryptophan+DMN+JC5519 prep	2.4
10 Tryptophan+DMN+JC5519 prep	1.0
11 Valine+DMN+JC5519 prep	1.1
12 Lysine+DMN+JC5519 prep	1.0
13 2-Aminofluorene+DMN+JC5519 prep	1.1

Experimental conditions as described in Table 1, except for the substitution of various amides instead of AAF.

**Fig. 3** Mutation ratio as a function of concentration. *a*, AAF held constant at 25 µg ml⁻¹ and DMN varied from 0.5 to 3.5 µg ml⁻¹. *b*, DMN held constant at 2.5 µg ml⁻¹ and AAF varied from 5 to 30 µg ml⁻¹. Procedure as given in Table 1.

series of other amides. Mutagen activity was observed with urea, a series of dipeptides and DMN in conjunction with the sonicated bacterial extract of JC5519, but no revertants were formed when amino acids or aminofluorene were substituted for AAF.

We believe that these observations may have importance in understanding the role of nitrosamines and the cause of cancer. *E. coli* is a common enteric bacteria, widely distributed in the environment and nitrosamines can be formed in a variety of circumstances. In the presence of amides of many types, highly reactive nitrosamides can be generated which may enter adjacent cells and cause mutation and possibly transformation to the neoplastic state. In this regard we have been able to detect in faecal flora the nitroso exchange reaction and we are exploring this reaction as a possible cause of colorectal cancer.

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Mast cell histamine and cell dehydration thirst

RAT mast cells, which synthesise and secrete the dipsogenic amines, histamine and 5-hydroxytryptamine (5-HT), are particularly abundant around small blood vessels and are known to be very sensitive to variations in tonicity¹. Hypotonic solutions cause their massive degranulation²⁻⁴, release amines from their granules^{5,6} and increase urinary histamine excretion⁷. Hypertonic solutions cause mast cell degranulation⁸ and shrinkage⁹. We report here evidence which suggests that the mast cell is an osmolar receptor and that histamine participates in the afferent link of the drinking behaviour elicited by hypertonic NaCl.

Male rats (300–320 g) were housed individually, and maintained on a 12-h dark-light cycle with continuous access to food pellets and tap water, except during the drinking tests when distilled water was used and food was removed. The drinking stimuli were the intraperitoneal (i.p.) injection of 3% NaCl (2.5 ml per 100 g)^{10,11} and the intravenous (i.v.) injection of 2 M NaCl (0.5 ml per 100 g)¹².

It is well known that the intraperitoneal injection of distilled water results in the depletion of mesenteric mast cells, whereupon they are replaced only slowly, regeneration becoming complete only about 6 weeks later^{4,13}. As can be seen in Fig. 1, the administration of 3% NaCl i.p. to rats injected 24 h earlier with distilled water (7.0 ml per 100 g i.p.) to deplete peritoneal mast cells, resulted in a marked reduction in their water intake during the first 2 h, although this difference was considerably reduced after 24 h (Table 1).

These data suggest that the peritoneal mast cell could be involved in the drinking response elicited by the intraperitoneal administration of 3% NaCl, but do not exclude the possibility that this is due to their anatomic disruption by the locally injected hypertonic solution.

The postulation of the peritoneal mast cells as an osmo-receptor requires the demonstration that peritoneal mast cell-depleted animals drink less in conditions of acute dehydration when the hypertonic NaCl load is administered by any other route.

Rats were anaesthetised with ether and the external jugular vein catheterised as described by Fitzsimons¹² for the intravenous injection of hypertonic solutions in unrestrained animals. When hypertonic NaCl was administered by this route, the drinking response was also significantly reduced in rats injected intraperitoneally with distilled water, 24 h earlier (Table 1). In fact, there were no differences between the amounts of water drunk by rats injected intraperitoneally or intravenously with hypertonic NaCl, either in the control or in the peritoneal mast cell-depleted groups.

Assuming that distilled water could alter other cellular structures in the mesentery besides disrupting the mast cells, we used disodium cromoglycate (DSCG), a drug that inhibits the release of histamine from the rat mast cell^{14,15}.

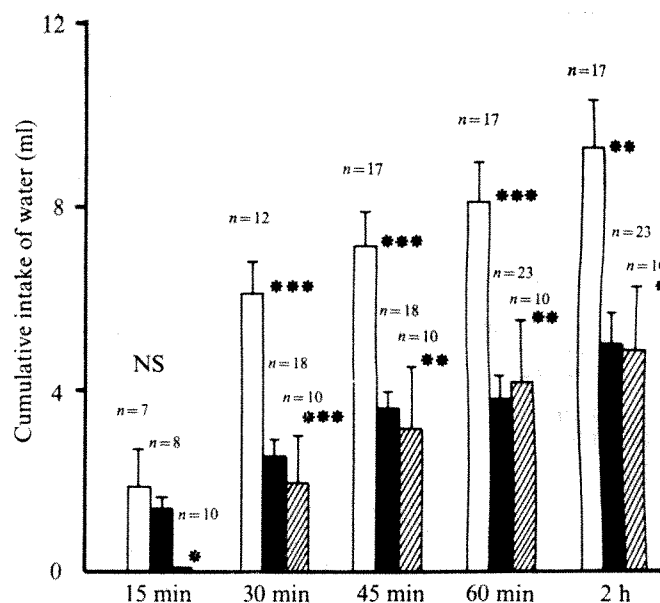


Fig. 1 Cumulative water intake ($\pm$ s.e.) for control rats (light bars), rats pretreated with intraperitoneal distilled water to deplete peritoneal mast cells (dark bars), and rats pretreated with DSCG (striped bars), after the injection of 3% NaCl 2.5 ml per 100 g i.p. * $P < 0.01$; ** $P < 0.005$; *** $P < 0.001$; **** $P < 0.0005$. The t test has been applied to control compared with distilled water and control compared with DSCG groups.

The administration of DSCG (95 mg kg⁻¹ i.p.) 5 min before injection of 3% NaCl i.p., resulted in a marked inhibition of drinking, of the same magnitude as that observed with the distilled water pretreatment (Fig. 1). As in the previous experiments, the differences were blurred after 24 h (Table 1). Increasing the dose of the drug (up to 190 mg kg⁻¹) did not result in further inhibition of drinking. Neither distilled water nor DSCG depressed or inhibited the drinking response to other dipsogenic stimuli (Table 2). These results indicate that the peritoneal mast cell is in fact involved in the detection of osmolarity changes in the body fluids.

5-HT exerts its dipsogenic effect through the release of kidney renin and the plasmatic generation of angiotensin¹⁶. As thirst elicited by hypertonic solutions is not mediated by the renal renin-angiotensin system^{10,11,17,18}, it seemed unlikely that mast cell 5-HT would be responsible for the drinking behaviour induced by highly concentrated NaCl solutions. As expected, pretreatment with the 5-HT antagonist, methysergide 2 mg kg⁻¹, which blocks 5-HT receptors without evidence of stimulation¹⁹, did not have any effect on the drinking response elicited by hypertonic NaCl (cumulative intake of water after 2 h: controls, 9.3 ± 1.0 , $n=17$; methysergide, 8.9 ± 0.5 , $n=4$).

On the other hand, intraperitoneally injected histamine induces drinking in intact and nephrectomised rats²⁰, which suggested that the hypertonic NaCl thirst could be mediated

Table 1 Effect of peritoneal mast cell depletion and DSCG on rats injected with hypertonic NaCl

	30 min	60 min	2 h	24 h
3% NaCl i.p.	6.43 $\pm$ 0.63 (22)	8.24 $\pm$ 0.66 (27)	8.98 $\pm$ 0.77 (27)	20.55 $\pm$ 2.17 (11)
2 M NaCl i.v.	8.05 $\pm$ 1.63 (10)	9.55 $\pm$ 1.58 (10)	10.30 $\pm$ 1.46 (10)	26.00 $\pm$ 3.65 (10)
DW + 3% NaCl i.p.	2.63 $\pm$ 0.28 (28)	3.65 $\pm$ 0.38 (33)	4.77 $\pm$ 0.52 (33)	18.20 $\pm$ 2.00 (21)
DW + 2 M NaCl (i.v.)	2.64 $\pm$ 0.88 (11)	3.05 $\pm$ 0.92 (11)	3.77 $\pm$ 1.04 (11)	20.91 $\pm$ 1.63 (11)
DSCG 95 mg kg ⁻¹ + 3% NaCl i.p.	1.95 $\pm$ 1.01 (10)	4.20 $\pm$ 1.39 (10)	4.95 $\pm$ 1.39 (10)	17.20 $\pm$ 3.60 (10)
DSCG 190 mg kg ⁻¹ + 3% NaCl i.p.	2.38 $\pm$ 1.00 (8)	4.75 $\pm$ 1.52 (8)	5.13 $\pm$ 1.40 (8)	16.13 $\pm$ 4.30 (8)

The amount of water drunk (mean $\pm$ s.e.) by rats injected intraperitoneally intravenously with hypertonic NaCl, pretreated with distilled water (DW, i.p.) to deplete mast cells, 24 h earlier, and with disodium cromoglycate (DSCG), 5 min earlier. For experimental details see text. Number of rats given in parentheses.

Table 2 Effect of distilled water, DSCG, and H₁ and H₂ antihistamines on the drinking induced by different thirst stimuli

Pretreatment	Thirst stimuli			
	24-h water deprivation (30 min CIW)	Isoprenaline 0.247 mg kg ⁻¹ s.c. (2 h CIW)	3% NaCl i.p. 2.5 ml per 100 g i.p. (2 h CIW)	Histamine 10 mg kg ⁻¹ i.p. (45 min CIW)
Control	8.5 ± 0.8 (10)	9.0 ± 1.0 (6)	8.9 ± 0.7 (27)	4.2 ± 1.2 (8)
DW 7.5 ml per 100 g i.p.	8.4 ± 0.3 (10)	7.5 ± 1.1 (12)	4.7 ± 0.5 (33)	6.8 ± 1.5 (5)
DSCG 95 mg kg ⁻¹	10.6 ± 1.1 (10)	8.9 ± 1.6 (5)	4.9 ± 1.3 (10)	6.1 ± 0.7 (8)
Diphenhydramine 40 mg kg ⁻¹	0 (10)	0 (10)	0 (10)	0 (10)
Mepyramine 32 mg kg ⁻¹	2.7 ± 1.4 (6)	2.3 ± 2.2 (4)	1.8 ± 0.8 (5)	0 (5)
Metiamide 50 mg kg ⁻¹	10.5 ± 1.0 (10)	7.2 ± 2.2 (7)	2.0 ± 0.4 (7)	1.0 ± 0.3 (7)

The cumulative water intake (mean ± s.e.) of rats pretreated with distilled water (DW) 24 h before the start of the water deprivation period, DSCG i.p. 5 min before, and diphenhydramine, mepyramine and metiamide, 10 min before the administration of the other stimuli and the availability of drinking water. Number of animals given in parentheses.

by histamine released from the peritoneal mast cells. The drinking induced by intraperitoneally injected histamine has been reported to be partially inhibited by the pretreatment with diphenhydramine 40 mg kg⁻¹, an antihistamine drug which blocks H₁ receptors²¹. When we pretreated with diphenhydramine 40 mg kg⁻¹ i.p. 10 min earlier, however, we completely abolished the water intake induced by 3% NaCl i.p., both in normal and nephrectomised animals, after 2 h, and markedly diminished the cumulative water intake after 24 h (Table 2). As it is well known that H₁ antihistamines also show anticholinergic activity^{19,22} and that diphenhydramine is the best known member of this group of drugs, we tried to discriminate between a specific antihistamine effect or a reduction of water intake due to the inhibition of cholinergic synthesis essential in the drinking response¹⁷. As can be seen in Table 2, diphenhydramine also abolished thirst induced by isoprenaline 0.247 mg kg⁻¹ subcutaneously, histamine 10 mg kg⁻¹ i.p., and water deprivation (24 h). These results showed that diphenhydramine is a nonspecific inhibitor of drinking in the rat.

Mepyramine 32 mg kg⁻¹, an H₁ antihistamine which is 40,000 times more active against histamine than acetylcholine²², reduced markedly and to the same extent the drinking induced by 3% NaCl, water deprivation (24 h),

histamine and isoprenaline (Table 2). These data, obtained with two H₁ antihistamines with different amounts of anticholinergic activity, suggested that either H₁ receptors were not involved in the histamine-induced thirst or that the administration of H₁ blockers was useless because they always inhibit drinking by a central effect.

We therefore tried the H₂ receptor blocker, metiamide, which shows practically no penetration into the central nervous system²³. As can be seen in Fig. 2, this H₂ antihistamine did not inhibit water intake induced by water deprivation (24 h) and isoprenaline, but blocked almost totally the drinking induced by 3% NaCl i.p. and histamine (Table 2).

Our experiments show that the reduction in the number of mesenteric mast cells and the blockade of mast cell secretion by DSCG reduce significantly the intake of water following the administration of hypertonic NaCl. This effect is most evident 2 h after the intraperitoneal or intravenous injection of the Na load, but is insignificant after 24 h. This was to be expected, as distilled water only destroys the mesenteric mast cells, leaving the rest of the mast cell complement of the body intact, and DSCG is rapidly eliminated¹⁴.

These experiments suggest that the mesenteric rat mast cell has a role in the detection of changes in tonicity of the body fluids. The mast cell is endowed with some of the characteristics that can be expected to be present in an osmotic receptor: perivascular distribution; high sensitivity to changes in tonicity; the ability to synthesise and release amines which induce drinking behaviour and a peculiar mechanism of secretion which involves the endocytosis of extracellular fluid²⁴⁻²⁶; and the eventual exchange of extracellular Na⁺ for histamine within the intracellular storage granules without exocytosis, as suggested by Foreman *et al.*²⁷.

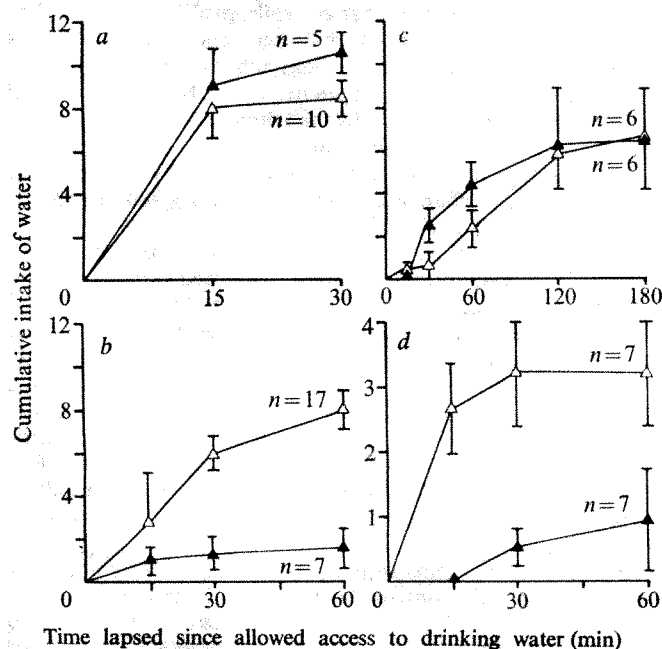
The inability of methysergide to inhibit thirst and its abolishment by metiamide in rats injected with 3% NaCl i.p. indicates that histamine is the mast cell amine involved in the triggering of the drinking response induced by a hypertonic load through the activation of an H₂ receptor.

Histamine induces thirst in the nephrectomised rat²⁰. This shows that the dipsogenic effect of histamine is not wholly mediated by the release of kidney renin and the plasmatic generation of angiotensin, and demonstrates the existence of another mechanism(s) able to convey to the central nervous system specific signals of thirst induction.

Studies using ¹⁴C-histamine have shown that it can cross the brain-blood barrier, even if in limited amounts²⁸. However small, these amounts of histamine might be of physiological importance, since, when applied directly into the hypothalamus, as little as 50 ng histamine can elicit a drinking response²⁹. The fact that metiamide, which does not cross the brain-blood barrier, inhibits both the drinking induced by histamine and hypertonic NaCl i.p., suggests that histamine triggers peripheral stimuli which reach the brain eliciting thirst.

Abdominal vagotomy produces severe deficits in drinking

Fig. 2 Influence of metiamide on the cumulative amount of water drunk by (a) rats deprived of drinking water for 24 h and after the injection of (b) 3% NaCl 2.5 ml per 100 g i.p.; (c) isoprenaline 0.247 mg kg⁻¹ s.c., and (d) histamine 10 mg kg⁻¹ i.p. The vertical bars are twice the s.e. Δ , Control; $\blacktriangle$, metiamide 50 mg kg⁻¹ i.p. 10 min before the rats were allowed access to drinking water (a) or 10 min before injection of the drugs (b, c and d).



behaviour in response to hypertonic NaCl, that are not reproduced by the administration of peripheral anticholinergic drugs³⁰. These data suggest that the critical lesion is the loss of afferent vagal stimuli. It is tempting to speculate that these vagal afferent stimuli are triggered by histamine, through an H₂ receptor. Experiments to test this hypothesis are in progress.

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Regulation of acid proteases during growth, quiescence and starvation in normal and transformed cells

CELL protein exists in a continual but variable state of turnover and the total protein content is the net result of the relative rates of synthesis and degradation. Cell growth is the net accumulation of protein, and although the relative contribution of each process is still uncertain the cessation of cell growth could be the result of a decrease in synthesis¹ and/or an increase in degradation². We report here that cessation of growth and starvation in normal cells is accompanied by a large increase in acid protease activity;

however, transformed cells which fail to stop growing also fail to elevate the enzymes responsible for hydrolysis of the great majority of peptide bonds.

When the culture medium is partially depleted of either essential nutrients³ or macromolecular serum growth factors⁴, the cell which retains its growth control will cease proliferation, and enter the G₀ (or extended G₁) phase of the cell cycle, where it can survive for prolonged periods in concentrations of these requirements less than those necessary for cell division. Some of the events which have been observed in response to deprivation of either nutrients or serum factors have been collectively called the 'pleiotypic response'⁵, and an analogous set of events in nutrient-deprived bacteria is known as the 'stringent response'⁶. Among the fundamental events in these starvation responses in both prokaryotes and eukaryotes is an increase in the rate of general cell protein degradation^{5,6}; however, the mechanisms of rapid and long term alterations in proteolysis have remained unknown. The function of the elevation in protein degradation during starvation is the maintenance of amino acid pools as a source of metabolic energy and as precursors for essential protein synthesis.

Whereas the rate of protein degradation is increased during cell starvation, there is now ample evidence that it is decreased during cell growth. As determined by the extracellular release of incorporated radiolabelled amino acids from cell protein, decreased proteolysis as well as increased synthesis contributes to the net gain of protein in several cell types, as observed *in vitro* and *in vivo*⁷. In contrast to the case of the increase in proteolysis during starvation, the cellular logic of the decrease during proliferation is uncertain, and space does not permit discussion of possibilities.

It is helpful for purposes of the present investigation to organise the above observations of the eukaryotic cell into three roughly distinguishable levels of protein degradation corresponding to three cellular conditions of proliferation, quiescence under adequate nutrition, and quiescence under starvation. The resting cell under adequate nutrition degrades its protein at a rate of approximately 2–3% h⁻¹ (ref. 7). Reports of this value, the quantitative extent of the decrease during proliferation and increase during starvation, vary considerably among different cells and tissues and are occasionally contradictory due in part to experimental difficulties in measuring protein degradation.

Figure 1 shows the simultaneous variation in cathepsin D activity, cell growth, and thymidine incorporation in 3T3 cells grown with and without changing the medium. When grown without medium change, cells proliferated logarithmically until d 4; growth then slowed; and at d 8 there was no increase in cell number. Changing the medium on d 4 caused the characteristic stimulation of DNA synthesis and cell growth shown in Fig. 1a, and final cell saturation density was six to seven times that of the unchanged cultures. Cathepsin D activity per cell in those cultures in which the fluid was not changed, increased 10–15-fold from its initial value in logarithmically growing cells and increased five to sixfold in those cultures in which the fluid was changed. Stimulation of growth caused the transient decline in protease activity shown in Fig. 1b, the extent of which depends on the persistence of the activity (enzyme half life) as well as the regulation of its synthetic rate. Thus, in the three different cellular condition of logarithmic growth, quiescence under adequate nutrition, and quiescence under a deficit of nutritional or hormonal factors, there are three different levels of the acid proteases (Fig. 1b). The identity of the factors depleted from the medium which cause the elevation in acid protease activity, is being investigated.

Those starving transformed cells which have completely lost their growth control, such as the Simian virus (SV)-transformed SV3T3 cell used in the present investigation, do

not undergo the same orderly alteration in diverse aspects of metabolism, which permits the quiescence and non-proliferative survival of normal cells or those tumourigenic cells which retain the capability to enter the G_0 phase. Instead, they proliferate suicidally; and, during the death of the culture, cells are distributed in all phases of the cell cycle⁸. (*In vivo* tumourigenicity probably involves factors in addition to alteration of proliferative controls.) The SV3T3 cell protease activity was very similar to that of the 3T3 during logarithmic growth; however, in complete distinction from the untransformed cell, the SV3T3 cell activity remained constant even into the death phase of the culture (Fig. 2). The SV3T3 cells did not secrete an appreciable fraction of the total content of the activity into serum-free medium during a period of 10 h; therefore, extracellular secretion is not a major route of turnover of this enzyme in this cell type. Thus, this highly transformed cell, which has lost the ability to enter G_0 in response to starvation, has also completely lost the ability to elevate the enzymes responsible for the bulk of intracellular protein degradation. The observed deficiency in protease regulation could be secondary to loss of some central control mechanism which also governs other aspects of metabolism ('unitary lesion') or alternatively could be an independent event.

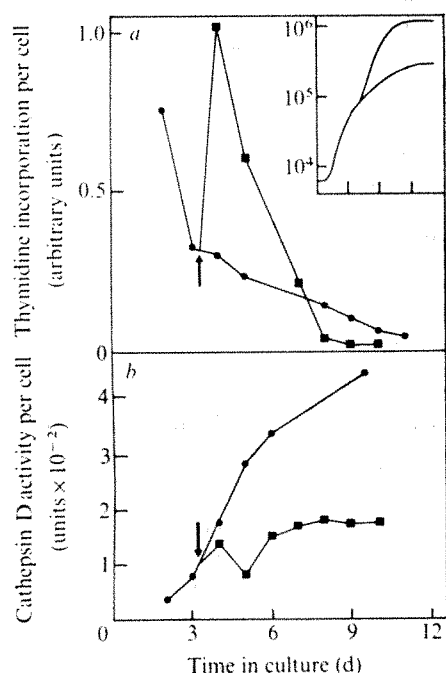


Fig. 1 Simultaneous variation in incorporation of thymidine per cell (a) and cathepsin D activity per cell (b) in BALB/c 3T3 cells compared with time in culture. 5×10^3 cells per 5-cm plate were seeded in Dulbecco-Vogt's modified Eagle's medium containing 10% foetal calf serum, and grown in a humidified incubator. At the time indicated by the arrow, the fluid of one group of plates was changed to fresh medium (■), whereas the other group was cultured in the original medium with no additions (●). At daily intervals, four plates of cells were counted with a Coulter counter (a, insert) and four plates were pulsed with ^3H -thymidine ($1 \mu\text{Ci ml}^{-1}$, $3 \mu\text{M}$, New England Nuclear, 6.7 Ci mmol^{-1}) for 2 h. Incorporated radioactivity per cell (a) was determined in washed 6% TCA precipitates (a). Cathepsin D activity (b) was determined by triplicate assay of the 0.1% Triton X-100 whole-cell homogenate pooled from four plates as described in Fig. 2. The decline in activity towards the initial value after fluid change was reproducible. Cell viability was 99% at day 10 as determined by Trypan dye exclusion. Increases in activity were somewhat greater when expressed as per unit of cell protein¹⁴.

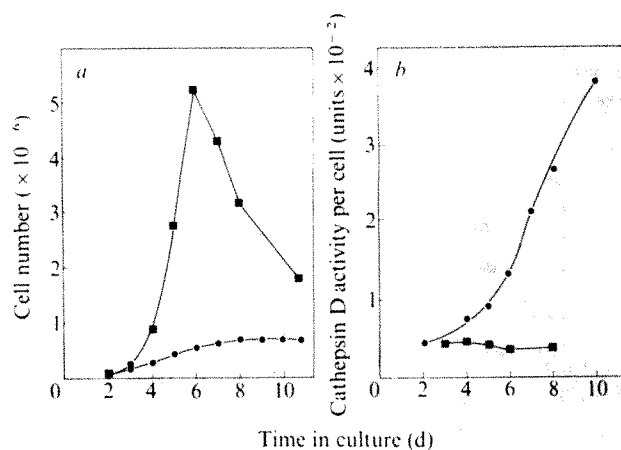


Fig. 2 Cell growth (a) and cathepsin D proteolytic enzyme activity per cell (b) in 3T3 and SV3T3 mouse fibroblasts, compared with time after plating. Both 3T3 and SV3T3 cells were seeded at 10^4 cells per 5-cm plate in the same batch of Dulbecco-Vogt's modified Eagle's medium containing 10% foetal calf serum, and grown without changing the medium. In these conditions the 3T3 cell populations (●) ceased proliferation and entered the G_0 phase of the cell cycle at 6–8 d, whereas the SV3T3 cells (■) continued to divide into the death phase (a). At daily intervals, three plates of cells were rinsed three times with phosphate-buffered saline and collected by scraping in 1 ml 0.1% Triton X-100. Three parallel cultures were counted with a Coulter counter. The cells were homogenised by passage through a fine gauge needle 10 times, and cathepsin D activity was determined in the whole-cell homogenate at pH 2.8 in 0.2 M citrate-phosphate buffer by TCA solubilisation of ^3H -haemoglobin¹⁵. A unit of enzyme activity is equivalent to $1 \mu\text{g}$ haemoglobin hydrolysed to 6% TCA soluble form per 10^6 cells per hour. At 10 d the viability of the 3T3 cells was greater than 99%. The slight decrease in SV3T3 activity per cell is due to the presence of 5–7% dead cells adhering to the monolayer, and when corrected accordingly, yields a constant value. Cathepsin D activity increased 12–15-fold in primary cultures of BALB/c embryonic fibroblasts and in Swiss 3T3 cells similar to that shown above for BALB/c 3T3 cells (b, ●), but remained constant in the BALB/c SV3T3 cell (b, ■) and Swiss SV3T3 cell. Culture in 0.2% serum also caused no elevation of cathepsin D activity in the SV3T3 cell (data not shown).

The profile of pH against protease activity which contains several proteases in addition to cathepsin D, seemed to be identical in logarithmically growing 3T3 and SV3T3 cells (data not shown). Furthermore, the entire pH against activity profile in 3T3 cells increased in a precisely constant proportion, indicating a regulatory alteration common to at least several acid proteases.

As observed in tissue culture, loss of mitotic control is not an all-or-none phenomenon, and diverse tumourigenic cell lines retain varying extents of the properties usually considered indicative of normal cells. For example, the HTC hepatoma cell under extreme nutritional deprivation exhibits both a G_0 arrest⁹ and an elevation in protein degradation¹⁰, whereas the SV3T3 cell used in these investigations undergoes neither³. Therefore, the regulation of lysosomal proteases was investigated in a benzo(a)pyrene-transformed cell line (BP3T3) which enters $G_{0,1}$ only under extreme serum deprivation^{11,12}. Accordingly it was found that these cells exhibited an intermediate degree of loss of protease control.

The quantitative relationship between alterations in cell content of acid proteases and corresponding alterations in the actual rate of protein degradation is currently unknown. An important precaution in the interpretation of the present data is the fact that control over intracellular protein degradation probably involves several factors in addition to the cell content of lysosomal proteases^{2,13}. Nevertheless, in conjunction with supportive studies of protein degradation, the pronounced elevation of lysosomal protease activities in untransformed cells on quiescence and starvation, and

regulatory deficiency of proteases in transformed cells, imply an involvement of these enzymes in the events of normal and altered proliferative control and altered proliferative control.

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Effect of hormones on herpes simplex virus type-2-induced transformation

GLUCOCORTICOID hormones enhance *in vitro* infections with murine leukaemia virus¹ and mouse mammary tumour virus². The yield of certain strains of herpes simplex virus type 2 (HSV-2) in certain cell types also increases in the presence of glucocorticoid hormones³. But in one limited study no significant differences were observed in the frequency of precancerous and cancerous lesions of the cervix in mice treated with oestrogens and HSV-2 or oestrogen alone⁴. Because there is epidemiological evidence that HSV-2 may be oncogenic in humans⁵, and experimental evidence that inactivated HSV-2 can produce malignant transformation of hamster embryo fibroblast cells *in vitro*^{6,7}, we have studied the effect of different hormones on HSV-2-induced transformation. We have used a rapid quantitative assay for transformation by HSV-2, using a thymidine kinase (TK) selection procedure described elsewhere (ref. 8 and unpublished work with N. Turner). These transformed cells have been shown to contain HSV-specific antigens as determined by indirect immunofluorescence, but do not yield infectious HSV. Our results indicate that 10^{-6} M cortisol and 10^{-8} M oestradiol inhibit HSV-2-induced transformation, and that dexamethasone has very little effect on the incidence of transformation.

A TK⁻ mouse cell line (designated N c1A c110) was obtained from Dr R. Goldberg of the National Institutes of Health and grown in 100-mm Petri dishes in Dulbecco modified Eagle medium supplemented with 10% foetal calf serum (FCS). HSV-2 strain 333 grown in Vero cells was used.

In transformation experiments, virus was ultraviolet-irradiated at $46 \text{ erg s}^{-1} \text{ mm}^{-2}$ and was mixed with trypsinised N c1A c110 cells (6×10^4 cells) at an unirradiated input multiplicity of 2.0 in 2 ml of Dulbecco medium. After adsorption for 1.5 h at room temperature, 15 ml of Dulbecco modified Eagle medium containing 0.16 mM thymidine, 0.05 mM adenosine, 0.1 mM glycine and 0.05 mM guanosine (TAGG medium) was added and the plates were incubated at 37 °C. After 72 h, the medium was removed and 15 ml of MTAGG medium (TAGG medium supplemented with 0.0006 mM methotrexate)⁹ was added. The cells were refed with MTAGG medium 5 d after the original feeding and weekly thereafter. Three weeks after infection, cultures

Table 1 Effect of hormones on HSV-2-induced transformation

Hormone treatment	Average no. of clones per plate	% of control
Untreated control	52	100
Cortisol, 10^{-6} M	10	20
Cortisol, 10^{-8} M	11	20
Cortisol, 10^{-7} M	15	22
Oestradiol-17 β , 10^{-8} M	8	15
Oestradiol-17 β , 10^{-6} M	40	80
Oestradiol-17 β , 10^{-7} M	45	90
Dexamethasone, 10^{-6} M	42	80
Dexamethasone, 10^{-8} M	52	100
Dexamethasone, 10^{-7} M	45	90

HSV-2-induced transformation was measured as described in the text. Hormones were added 4 h after the addition of irradiated virus and remained present for 21 d. Number of clones per plate is average of 4 replicate plates. Mock-infected N c1A c110 cells did not develop clones.

were fixed with 5% formalin and stained with a 1% aqueous solution of crystal violet. Hormones (Sigma) were dissolved in absolute alcohol and added to the medium in appropriate final concentration.

The HSV-2-induced transformation was carried out in the presence of cortisol, oestradiol-17 β or dexamethasone. Table 1 shows a fivefold decrease in the number of transformed clones that developed in the presence of cortisol (10^{-6} to 10^{-7} M). Oestradiol considerably inhibited the number of transformed clones at a concentration of 10^{-8} M, but had no effect at concentrations of 10^{-6} M or lower. In contrast, dexamethasone had very little effect on the number of transformed clones at all concentrations (10^{-8} to 10^{-7} M) examined. Pretreatment of cells with hormones before infection and continuation of hormone treatment after infection (data not shown) did not alter the results described in Table 1.

To determine whether the observed inhibitory effect of cortisol and oestradiol is due to the toxic effect on host cells, N c1A c110 cells were treated with cortisol and oestradiol. After incubation at 37 °C for 48 h, cell numbers and DNA and protein synthesis were measured and compared with untreated controls. Because these cells are deficient in TK, DNA synthesis was measured by determining the incorporation of ³H-hypoxanthine into alkaline-trichloroacetic acid (TCA)-insoluble material. Table 2 shows that cell numbers, DNA and protein synthesis were not affected by treatment with cortisol or oestradiol at a concentration which inhibited HSV-2-induced transformation. This is further supported by the observation that pretreatment of cells with hormones before the addition of irradiated virus had no inhibitory effect on HSV-2-induced transformation (Table 3).

To determine whether the presence of hormones is

Table 2 Effect of hormones on cell growth and DNA and protein synthesis

	Cell no.* per plate ($\times 10^4$)	DNA synthesis† ³ H-hypoxanthine incorporated (c.p.m.)	Protein synthesis† ³⁵ S-methionine incorporated (c.p.m.)
Untreated control	4.80	23,840	59,451
Cortisol, 10^{-6} M	4.89	21,486	61,118
Oestradiol, 10^{-8} M	4.35	22,576	62,198

*Hormones were added 6 h after seeding the N c1A c110 cells into 60-mm Petri dishes. Two days later, both treated and untreated cultures were trypsinised and counted. Number represents average of two replicate plates.

†Hormones were added as described above. One day later, ³H-hypoxanthine and ³⁵S-methionine were added to both untreated and hormone-treated cells to a final concentration of $1 \mu\text{Ci ml}^{-1}$ and $2 \mu\text{Ci ml}^{-1}$, respectively. After incubation for 48 h at 37 °C, ³⁵S-methionine-labelled cells were lysed with 2% sodium dodecyl sulphate and TCA-precipitable radioactivity was determined. Hypoxanthine-labelled cells were lysed by 0.4% Triton X-100 and digested with 0.3 N KOH at 37 °C overnight, after which TCA-precipitable radioactivity was determined.

necessary throughout the 21 d of incubation, cortisol (10^{-6} M) was kept in the medium for various times and its effect on the number of transformed clones was examined. Data indicate that the presence of cortisol was necessary only for the first 2 d after infection to exhibit inhibitory effect, although inhibitory effect is maximum between 2 and 4 d after addition of virus.

We have examined the possibility that the observed inhibition of transformation is somehow related to a more generalised inhibition of herpes virus development in productive infection. Table 4 indicates that cortisol (10^{-6} M) and oestradiol (10^{-8} M) had no effect on the formulation of herpes virus progeny or on viral DNA synthesis.

Our results demonstrate that HSV-2-induced transformation is inhibited in the presence of cortisol (10^{-6} to 10^{-7} M) and oestradiol (10^{-8} M). In contrast, dexamethasone has very little effect on HSV-2-induced transformation. We do not know why dexamethasone and cortisol, both being glucocorticoid hormones, have different effects on transformation. The difference could be due to the differences in cell receptor for the natural (for example, cortisol) and synthetic (for example, dexamethasone) hormone.

Table 3 Effect of cortisol on HSV-2-induced transformation at various times after infection

Cortisol present (d)	No. of transformed clones per plate
None	55
-2 to 0	50
0 to 2	18
0 to 4	11
0 to 10	10
0 to 21	11
10 to 21	56

Transformation was carried out as described in the legend to Table 1. The interval in which cortisol was present is indicated, zero being the day of virus addition. Twenty-one days after infection, transformed clones were stained and counted. Number of clones per plate is the average of four replicate plates.

Hormones were added 3-4 h after the virus in the transformation assay. We therefore concluded that the inhibition of transformation by hormones is probably not due to the failure of adsorption, penetration or uncoating of infecting virus particles. This was further supported by the fact that the inhibition of transformation was still observed when hormones were added 6-7 h after addition of virus (data not shown). We do not know whether parental viral DNA undergoes replication in the transformation process. Even if the viral DNA is replicated during transformation, this replication probably would not be inhibited by cortisol or oestradiol, because these hormones have no inhibitory effect on viral DNA synthesis in productive infection (Table 4). Because the addition of cortisol at late infection (10 d after infection) did not inhibit transformation (Table 3), it may be concluded that hormones probably do not act at the level of transcription or translation of the virus gene. One possible explanation is that hormones inhibit the integration of HSV-2 DNA into the cellular genome. In the absence of permanent association of viral DNA with the cellular genome, the genetic information for TK is not transferred to the daughter cells, and therefore the formation of transformed clones is inhibited. The mechanism by which hormones might inhibit integration of viral genes into the cellular genome is unknown. This may inhibit the steps involved in integration, for example, specific cleavage of the cellular DNA, circularisation of virus DNA, or joining of virus DNA with cellular DNA. The role of hormones in virus integration has not been reported, but this study may suggest a potential use of hormones to study this

Table 4 Effect of hormones on the synthesis of virus progeny and virus DNA in HSV-2-infected N c1A c110 cells

	Virus yield* (PFU per plate)	DNA synthesis† ³ H-thymidine incorporated (c.p.m.)
Untreated control	4.5×10^6	4.2×10^4
Cortisol 10^{-6} M	4.2×10^6	9.0×10^5
Oestradiol 10^{-8} M	4.3×10^6	3.8×10^4

*Confluent monolayers of N c1A c110 cells were infected with HSV-2 strain 333 at an input multiplicity of 1. After adsorption of virus for 1 h at 37 °C, Dulbecco's modified Eagle medium containing 5% FCS was added. At 24 h after infection, cells were scraped into the medium and the mixture was frozen and thawed three times. After centrifugation at 1,000 r.p.m. for 5 min, the supernatant was assayed for viral progeny in rabbit kidney cells by plaque assay. Numbers represent average of two plates.

†Confluent cells were infected with HSV-2 at an input multiplicity of 5 as described above. At 7 h after infection, ³H-thymidine was added to a concentration of 5 μ Ci ml⁻¹. After 24 h of infection, labelling medium was removed, cells were lysed by 2% sodium dodecyl sulphate and TCA-precipitable radioactivity was determined. Because the CsCl density gradient analysis of labelled DNA synthesised after 7 h of infection is mostly virus specific (our unpublished results), incorporated radioactivity represents virus-specific DNA.

phenomenon. We do not have any direct information about the presence of hormone receptors in N c1A c110 cells, but in conjunction with the accepted mode of action of steroid hormones, we can assume that in this case hormones are also acting through specific cell receptors. There are several possible reasons why we did not see any significant effect of hormones in the productive system. The frequency of integration during the productive infection is presumably very low and any effects would be minimal. Also, productive infection with HSV may change the receptor level in the cells, thereby rendering infected cells insensitive to hormones.

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Cytoskeletal regulation of concanavalin A capping in pulmonary alveolar macrophages

CONCAVALIN A (con A) capping occurs in polymorphonuclear leukocytes (PMN) and is thought to be dependent on disruption of microtubules with subsequent lateral movement of lectin receptors to a polar region in the plasma membrane¹. Capping in PMN is induced by colchicine but is blocked by cytochalasin B, an inhibitor of microfilament assembly². Based on these observations, a cytoskeletal system that effects transmembrane control of receptor mobility has been proposed. The microtubules system interacts directly or indirectly with membrane components to limit the

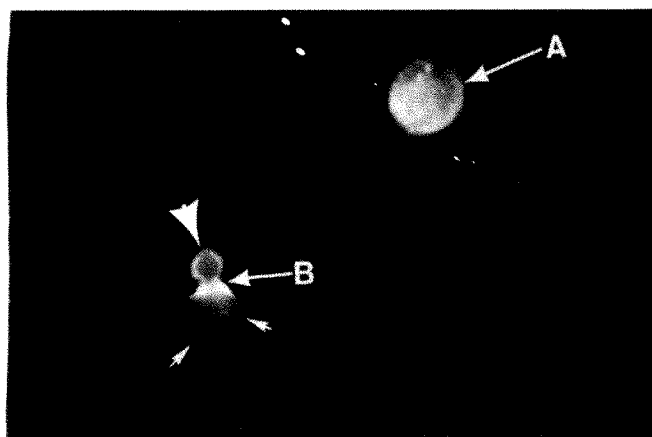


Fig. 1 Photomicrograph of alveolar macrophages showing diffuse fluorescence (A) and polarised fluorescence pattern (cap) (B). Small arrowheads show cell outline, large arrowhead points to fluorescent uropod cap. ($\times 400$.)

mobility of surface receptors while the contractile system of microfilaments may enhance the movement of these receptors into caps³. Here we report that capping also occurs in guinea pig alveolar macrophages (AM), and that the factors which regulate AM surface receptor movement differ dramatically from those in PMN. AM are capped by cytochalasin B alone, which is contrary to the experience with the PMN. AM capping is dependent on oxidative phosphorylation and the cap is associated with formation of a striking uropod (Fig. 1).

Guinea pig AM were obtained using the method of Rister and Baehner⁴, in Krebs-Ringer-phosphate (KRP) buffer with 5 mM glucose. Fluorescein-conjugated con A (FITC-con A, Yeda) was suspended in normal saline. AM were incubated for 2 min at 37 °C; after addition of appropriate drugs (Table 1) for 15 min, AM were labelled with FITC-con A for 5 min at 37 °C, then fixed for 10 min at 28 °C with formaldehyde and centrifuged for 10 min. Slides were prepared and capped cells counted.

Table 1 shows the response of AM to various drugs. Colchicine-induced capping was not inhibited by pretreatment of AM with cytochalasin B ($10 \mu\text{g ml}^{-1}$). Cytochalasin B treatment ($1-20 \mu\text{g ml}^{-1}$) induced an equivalent degree of capping as noted with colchicine. Dimethyl sulphoxide (DMSO), the solvent for cytochalasin B had no effect on

Table 1 Effect of various additions on concanavalin A capping in pulmonary alveolar macrophages

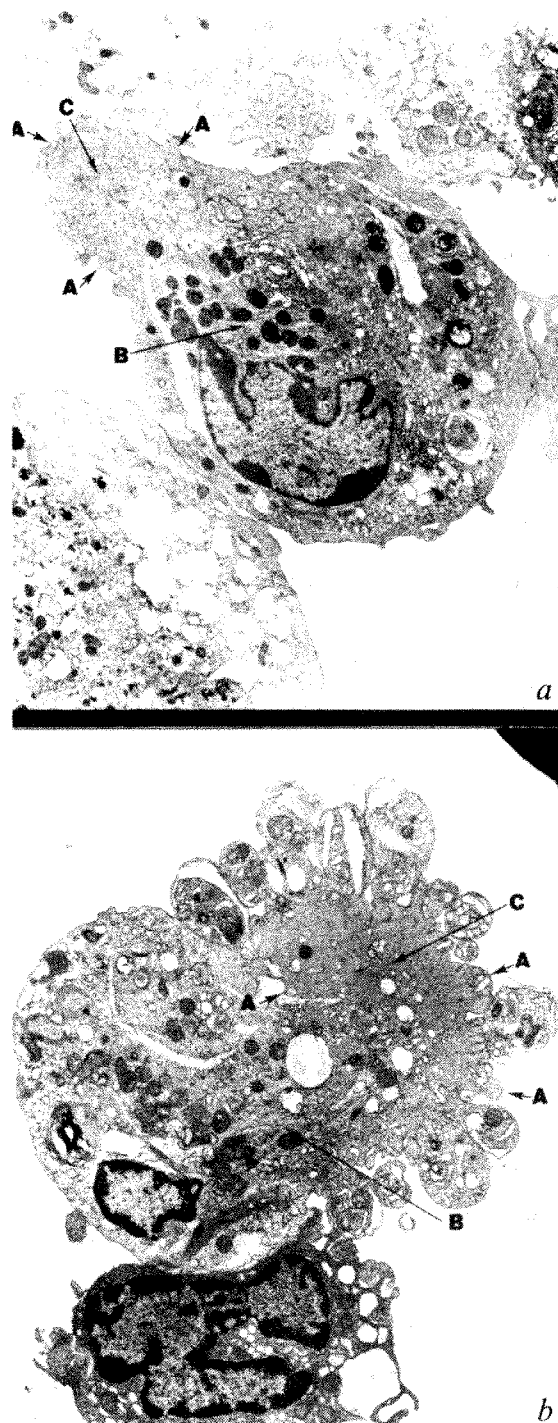
Addition	% Capped
None (control)	31.6 ± 8.5
Colchicine (10^{-5} M)	63.0 ± 7.7
Colchicine (10^{-5} M) + cytochalasin B ($10 \mu\text{g ml}^{-1}$)	78.0 ± 0.9
Cytochalasin B	
1 $\mu\text{g ml}^{-1}$	70.5 ± 4.3
5 $\mu\text{g ml}^{-1}$	78.6 ± 7.3
20 $\mu\text{g ml}^{-1}$	73.2 ± 14.6
DMSO 300 $\mu\text{g ml}^{-1}$	18.2 ± 6.3
Colchicine (10^{-5} M) at 4 °C	0 0
then warmed to 37 °C	66, 65
Cytochalasin B ($10 \mu\text{g ml}^{-1}$) at 4 °C	0 0
then warmed to 37 °C	70, 73
Potassium cyanide (1 mM)	0 0
2-Deoxyglucose (1 mM)	31, 34

Alveolar macrophages (2×10^6 cells) were incubated at 37 °C in a shaking water bath in KRP buffer, 3 ml total volume, containing 5 mM glucose with drugs indicated. After 15 min cells were stained with FITC-con A ($50 \mu\text{g}$) at 37 °C for 5 min, then fixed with 2% formaldehyde for 10 min at 28 °C. Cells were then washed once and centrifuged and slides made from the cell button. Results were obtained by examining 200 cells per slide at random with a Zeiss fluorescence microscope (FITC-exciter filter, 530 barrier filter) and classifying each cell as capped or not capped. Values represent means $\pm$ s.d.

capping. Capping did not occur at 4 °C although warming the AM suspension to 37 °C for 15 min produced maximal capping. AM treated for 45 min with potassium cyanide did not cap, whereas incubation with 2-deoxyglucose, an inhibitor of anaerobic glycolysis⁵ had little effect on capping indicating a dependence on energy derived from oxidative phosphorylation.

Transmission electron microscope studies were carried out to determine the intracellular location of microfilaments and microtubules in capped cells (Fig. 2). In cells treated with colchicine (Fig. 2a), few microtubules were visualised and the cap contained an abundance of microfilaments. In

Fig. 2 Transmittance electron micrograph of alveolar macrophages *a*, capped after treatment with colchicine in which microtubules are not apparent, and *b*, after cytochalasin B. A, Outlines the region of cell cap; B, 100 Å filaments; C, microfilament rich region in cap. ($\times 7,000$.)



cells treated with cytochalasin B (Fig. 2b), the caps were surrounded by typical cytochalasin B polarised blebs⁶, but the caps in these cells were also rich in microfilaments.

This study shows that con A capping in the AM can be induced by inhibitors of both microtubule and microfilament assembly. Thus in AM, some microfilaments and microtubules do not seem to have opposing roles in controlling membrane fluidity, as some recent theories propose for other cell types³. Instead, it seems that in the AM, redistribution of lectin receptors, as occurs in capping, could be dependent on a set of microfilaments that are not sensitive to cytochalasin B. Other studies have proposed such a set of microfilaments in the AM (ref. 7). The receptor mobility in the AM may be limited by both the microtubules, as in other cell types, and a subset of microfilaments sensitive to cytochalasin B.

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α -Fetoprotein induces suppressor T cells *in vitro*

THE ability of α -fetoprotein (AFP) of mouse or human origin to suppress certain T cell-dependent immune reactions *in vitro* is well documented¹⁻⁴. These findings on a tumour-associated embryonic substance⁵ have potentially important implications as to our understanding of the maternal-foetal immunological relationship⁶, the development of immune capabilities in the foetus and newborn⁷, and of certain diseases⁸ where immunological hyporeactivity and elevations in AFP often occur concomitantly. The mechanism(s) through which AFP is exerting its immunoregulatory influence is largely unknown; however, previous studies¹ have shown that AFP must be added at the initiation of antigen-stimulated spleen cell cultures for maximal inhibition of primary antibody synthesis to occur.

Continued presence of AFP in the cultures for 8-12 h was sufficient to maintain suppression in an AFP-free environment for at least 5 d *in vitro*¹ and 10 d *in vivo* using adoptive transfer experiments (unpublished). These observations suggested that AFP may inhibit immune responses indirectly by activating regulatory suppressor cells. Here we report that AFP does indeed induce the formation of highly efficient suppressor T cells with capacity to inhibit helper T cells, but with no effect on B cells responding to thymus independent antigens.

Mouse amniotic fluid (MAF) was collected from pregnant outbred Swiss mice (Anticimex, Stockholm) between day 14 and day 16 of gestation. AFP was isolated from MAF by a two-step procedure of affinity chromatography and preparative polyacrylamide gel electrophoresis as described before¹. Purity was checked by gel electrophoresis and immunological tests. In initial experiments normal adult C57BL/6 or BALB/c spleen cells were cultured in Marbrook chambers⁹ with purified AFP, MAF or normal mouse serum (NMS) at equivalent final concentrations in the cultures of 200 μ g ml⁻¹ for 4 d. There were no significant differences in the yields of viable lymphocytes from cultures containing AFP, MAF, and NMS. The precultured cells were then washed, and 10⁶ cells were added to 20 $\times$ 10⁶ fresh normal spleen cells in new Marbrook cultures along with an optimal immunising dose of sheep red blood cells (SRBC). After 4 d in culture the cells were harvested and assayed for primary plaque-forming-cells (PFC) to SRBC. The results of three such experiments are summarised in Table 1. In all experiments the addition of spleen cells precultured in MAF or MAF-derived AFP to assay cultures caused significant suppression of the PFC response to SRBC.

The time-course of development of suppressing ability was determined by assaying for suppressor cell activity after various preculture times ranging from 6 to 96 h. Because of the limited amount of purified AFP available, these and subsequent experiments to be described in this report were performed with MAF. It is justified in this system to attribute the resulting effect to AFP since the previous experiments (Table 1) showed that equivalent suppressor cell activity is obtained by preculturing with AFP and MAF. It has also been clearly demonstrated¹ that the factor in MAF responsible for its suppressive effect on T cell-dependent immune responses is AFP. Although some suppression was evident after 2 d of preculture, 3-4 d were required for maximum development of suppressor cell activity (Table 2). This suggests that AFP is actively generating suppressor cells through a process involving cell proliferation and/or differentiation. In line with this are findings in another test system that AFP under certain culture conditions will induce proliferation of normal mouse T lymphocytes (A. B. Peck, R. A. M. and H. W., in preparation).

Table 1 Suppression of the primary PFC response to SRBC by spleen cells precultured in AFP

Preculture* Cells precultured in:	No. of precultured cells added	Assay culture† γ M PFC per culture (% suppression)		
		Experiment 1	2	3
NMS	10 ⁶	1,800	1,640	900
AFP	10 ⁶	2,340 (0)	2,000 (0)	1,116 (0)
MAF	10 ⁶	850 (53)	510 (69)	370 (59)
		1,000 (46)	420 (74)	573 (36)

*20 $\times$ 10⁶ normal mouse spleen cells were precultured with 200 μ g ml⁻¹ NMS (normal mouse serum), AFP (α -fetoprotein) or MAF (mouse amniotic fluid) in Marbrook chambers⁹ using RPMI 1640 medium supplemented with 10% foetal calf serum, 10 mM HEPES and 5 $\times$ 10⁻⁵ M 2-mercaptoethanol. After 4 d of incubation at 37 °C in 5% CO₂ humid atmosphere, the cells were collected and viable cells were counted by a dye exclusion method.

†10⁶ Viable lymphocytes precultured as indicated above were added to assay cultures of 20 $\times$ 10⁶ fresh normal spleen cells stimulated with 3 $\times$ 10⁶ SRBC in Marbrook chambers⁹. One set of control cultures consisted of fresh spleen cells and antigen without added precultured cells. Direct anti-SRBC PFC were assayed after 4 d in culture². The data from three representative experiments are expressed as arithmetic means of three cultures per group.

Table 2 Time course of development of suppressor cells *in vitro*

Preculture*		Assay culture†	
Cells precultured in:	Duration of preculture (h)	γ M PFC per culture	% Suppression
NMS	6	935 $\pm$ 45	6
MAF		1,019 $\pm$ 214	0
NMS		1,171 $\pm$ 158	0
MAF	12	1,197 $\pm$ 145	0
NMS		1,092 $\pm$ 73	0
MAF		1,095 $\pm$ 125	0
NMS	24	887 $\pm$ 174	11
MAF		638 $\pm$ 89	36
NMS		918 $\pm$ 56	8
MAF	48	560 $\pm$ 65	44
NMS		1,000 $\pm$ 91	0
MAF		372 $\pm$ 41	63

*Preculture conditions were as described in the legend to Table 1. The duration of the precultures varied from 6–96 h as indicated.

† 10^6 Viable lymphocytes from preculture were added to assay culture stimulated with SRBC as described in Table 1. Control cultures (cells precultured in NMS) in the experimental group receiving 96 h precultured cells were arbitrarily adjusted to 1,000 PFC per culture. All other values were normalised to these control cultures. Each value represents the mean and s.e.m. of triplicate cultures.

Results presented in Table 3 suggest that the suppressor cell activity induced by AFP is mediated by T lymphocytes. Here the degree of suppressor cell activity generated in precultures of unfractionated spleen cells was compared with that generated by splenic T cells isolated by affinity fractionation on glass bead columns¹⁰. There was a significant enrichment of suppressor activity in precultures of purified T cells, whereas suppressor activity was absent in precultures of spleen cells from nude (*nu/nu*) mice with a BALB/c background, but present in precultures of normal BALB/c spleen cells (Table 3).

A dilution analysis of the suppressor T-cell activity was then performed by adding graded numbers of precultured cells to assay cultures of 20×10^6 normal spleen cells which were then challenged with SRBC or with the thymus independent antigen DNP₃₂-Ficoll¹¹ (DNP, 2,4-dinitrophenyl). and assayed for PFC to SRBC or TNP (2,4,6-trinitrophenyl). A linear dilution effect of AFP-induced suppressor cell activity on SRBC PFC responses was obtained within the range of 10^7 – 10^4 added cells as shown in Fig. 1b. As few as 10^4 suppressor cells (0.05% of total cell population) markedly inhibited T cell-dependent anti-SRBC responses. There was, however, no effect of suppressor cells on the antibody response to DNP₃₂-Ficoll (Fig. 1a). A similar pattern of suppression of T cell-dependent antibody responses

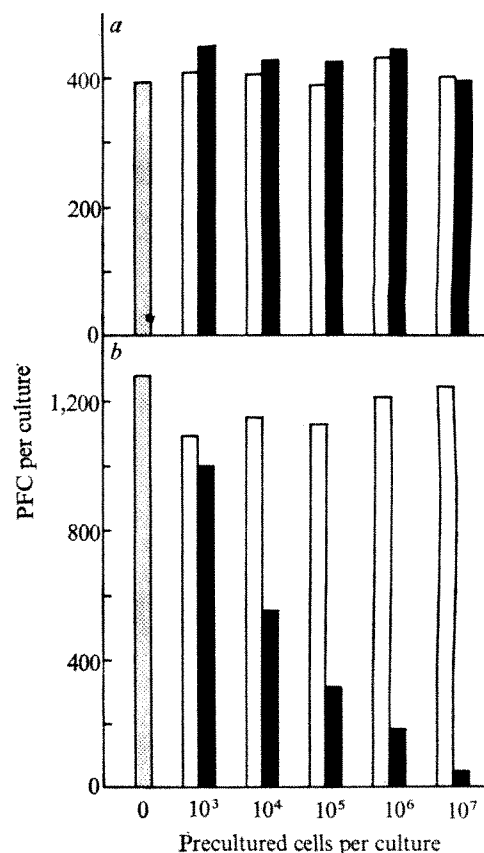


Fig. 1 Effects of various numbers of precultured cells on the primary *in vitro* antibody response to $10 \mu\text{g ml}^{-1}$ DNP₃₂-Ficoll (a), and 3×10^6 SRBC (b). The abscissa denotes the number of viable precultured cells added to standard assay cultures. Grey bars refer to antigen-stimulated control cultures without added precultured cells. Open bars refer to cells precultured in NMS and black shaded bars refer to cells precultured in MAF. The thymus-independent PFC response to DNP₃₂-Ficoll¹¹ was assayed against TNP-SRBC².

to DNP-KLH, and a lack of inhibition of T cell-independent antibody responses to DNP-POL has been recorded in several, additional experiments.

Thus our results strongly suggest that one mode of immunoregulatory action by AFP is the generation of suppressor T cells which control the level of responsiveness to T cell-dependent antigens. This is quite in agreement with the findings that in newborn mice with high intrinsic concentrations of AFP there exist efficient numbers of sup-

Table 3 Evidence that T cells mediate suppressor activity *in vitro*

Cell source	Preculture* Cells precultured in:	No. of precultured cells added	Assay culture† IgM PFC per culture	% Suppression
C57BL/6 whole spleen cells	NMS	10^6	1,000†	—
C57BL/6 whole spleen cells	MAF	10^6	990 $\pm$ 50	1
C57BL/6 column Purified T cells	NMS	10^6	453 $\pm$ 17	55
C57BL/6 column Purified T cells	MAF	10^6	981 $\pm$ 32	2
BALB/c spleen	NMS	10^6	52 $\pm$ 8	95
BALB/c spleen	MAF	10^6	1,000†	—
<i>nu/nu</i> spleen	NMS	10^6	1,116 $\pm$ 50	0
<i>nu/nu</i> spleen	MAF	10^6	411 $\pm$ 78	59
	NMS	10^6	1,174 $\pm$ 117	0
	MAF	10^6	1,117 $\pm$ 61	0

*Normal spleen cells, Ig-anti-Ig column purified splenic T cells¹⁰, or spleen cells from thymus-deficient (*nu/nu*) mice were precultured in NMS or MAF for 4 d as described in Table 1.

†The SRBC PFC responses in control cultures of fresh normal spleen cells without added precultured cells were adjusted to 1,000 and experimental values were normalised as described in Table 2.

pressor T lymphocytes^{12,13}. Also, it is known that thymus-derived lymphocytes from newborn humans can function as suppressor cells with regard to the proliferative capacities of the lymphocytes from their mothers (ref. 14 and L. B. Olding, R.A.M. and H.W., in preparation). We therefore suggest that AFP serves as an agent to protect the mammalian foetus from graft-versus-host and host-versus-graft reactions through the selective induction of suppressor T cells. The exact mechanism(s) through which AFP is exerting this activity is now a major target of analysis.

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Unmasking of thromboxane A₂ synthesis by ureteral obstruction in the rabbit kidney

THE rabbit kidney has a high capacity for prostaglandin biosynthesis. The major renal prostaglandin released in response to various stimuli is PGE₂, a vasodepressor prostaglandin¹ thought to have a role in the regulation of intrarenal blood flow². Although renal prostaglandin synthesis may initiate a reduction in vascular resistance of the kidney in response to reduced perfusion pressure, it has not been possible to establish a functional role for renal prostaglandins as mediators of increased vascular resistance evoked by augmented renal perfusion pressure. An increase in renal resistance, if related to changes in the rate of synthesis of endogenous substances, implies the biosynthesis of a vasoconstrictor or suppression of the basal production of a vasodepressor material. Unilateral ureteral obstruction (model of hydronephrosis) in the dog, rat and rabbit is initially associated with a decrease in renal resistance which is followed by a fall in GFR and renal blood flow^{3,4} by 24 h post-occlusion. The initial renal vasodilator phase may be associated with enhanced renal basal PGE₂ synthesis and markedly facilitated PG release in response to hormone stimulation⁵. Here we report the observation that the ureter-obstructed kidney may be producing a natural vasoconstrictor material.

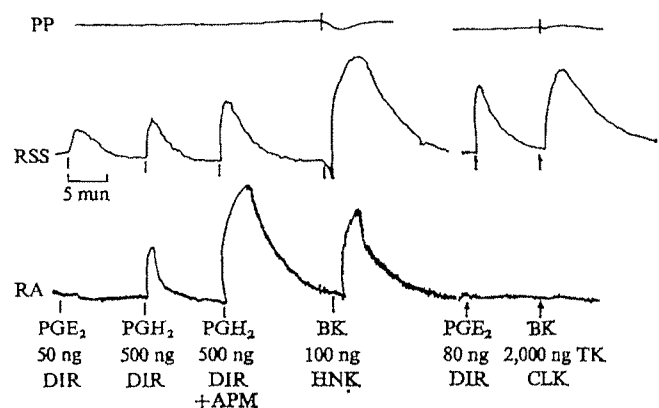
New Zealand male white rabbits were anaesthetised with pentobarbital and the left ureter was ligated⁶. Three days later the rabbits were anaesthetised and both kidneys (ipsilateral ureter obstructed and contralateral untouched kidney) were removed and perfused (15 ml min⁻¹) with Krebs-Henseleit buffer at 37 °C as previously described⁵. The venous effluent was allowed to flow over a series of biological assay organs (rabbit aorta, chick rectum, rat stomach) which were selected to

detect the presence of PGE, PG-endoperoxides and thromboxane-like substances (details Fig. 1 legend).

The isolated perfused rabbit kidney, with the ligated ureter, released into the venous effluent a substance which contracted the rabbit aorta following the administration of a dose of bradykinin (100 ng) (Fig. 1). The contralateral kidney did not release the vasoconstrictor into the effluent even with very high doses of bradykinin (2,000 ng). Alterations in bradykinin metabolism were not enough to explain differences in release from hydronephrotic and contralateral kidney⁶. Indomethacin, a known inhibitor of the cyclo-oxygenase⁸, abolished the generation of the aortic constrictor by the ureter obstructed kidney in response to bradykinin. The PG products of cyclo-oxygenase known to constrict the rabbit aorta are the endoperoxides, PGH₂ and PGG₂ (half life 6–7 min at 37 °C) and thromboxane A₂ (half life 30 s)^{9,10}. The endoperoxides, which are moderately potent aorta vasoconstrictors, are rapidly converted to the classical prostaglandins (PGE₂, PGD₂) by enzymic and non-enzymic breakdown. The enzyme thromboxane synthetase converts the endoperoxide PGH₂ to the potent constrictor thromboxane A₂ and has been demonstrated in platelets⁹, spleen¹⁰ and lung¹¹. Thromboxane synthetase has not been described in the kidney.

To ascertain which of these two prostaglandin constrictors was being produced in the hydronephrotic kidney, the half-time of disappearance of the constrictor substance from the aqueous media was estimated by bioassay. The venous effluent released in response to hormone stimulation was collected, reoxygenated and reperfused over a second aortic strip after a delay of 1.5 min. The original effluent contains both PGE₂ (that is contraction of chick rectum and rat stomach) which is stable in aqueous solution at 37 °C and the rabbit aorta constricting substance (Fig. 2). Following the 1.5 min delay the PGE₂ activity was still present but the constrictor substance had completely disappeared. PGH₂ injected directly over the strips and subjected to the same delay of 1.5 min still produced contraction of the aorta. This instability in aqueous solution suggested that the constrictor was the more labile thromboxane A₂ and not the endoperoxide PGH₂. The renal release of rabbit aorta constrictor activity was dose dependent and was also released from the hydronephrotic kidney following stimula-

Fig. 1 The release of a rabbit aorta contracting substance from a ureter obstructed kidney by bradykinin. The venous effluent from the perfused rabbit kidney is allowed to superfuse a rabbit aortic strip and a rat fundal strip. Sensitivity and selectivity of the assay tissues were increased by an infusion of antagonists⁶ to histamine, acetylcholine, catecholamines and 5-hydroxytryptamine as well as indomethacin (1 µg ml⁻¹) to prevent endogenous synthesis of prostaglandins by the tissues⁷. PP, renal artery perfusion pressure; RSS, rat (fundic) stomach strip; RA, rabbit aortic strip; DIR, injection directly over strips; TK, through the kidney; BK, bradykinin; PGH₂, endoperoxide; HNK, hydronephrotic or ureter obstructed kidney; and CLK, contralateral or untouched kidney (n = 10).



tion with angiotensin II 50 ng but not from contralateral kidney with doses up to 1,000 ng (data not shown).

Microsomal fractions from kidney homogenates were prepared for the radiochemical verification of thromboxane synthesis in the hydronephrotic kidney. The kidneys were minced and homogenised (Polytron) in 3 volumes of 100 mM phosphate buffer pH 7.5. The homogenate was centrifuged (8,000g, 15 min) and the supernatant was centrifuged at 100,000g for 60 min. The pellet was resuspended in 100 mM phosphate buffer pH 7.5 in a volume equal to one fourth original wet weight of the tissue. The kidney microsomes (2 mg protein per ml) were incubated with ^{14}C -arachidonic acid in the presence of 1.2 mM L-adrenaline¹³ for 30 min at 37 °C and the volume of the reaction mixture was 1 ml. The reaction was acidified to pH 3.5 with citric acid and extracted twice with 2 volumes of ethyl acetate. The ethyl acetate phase was evaporated to dryness and reconstituted in chloroform-methanol (2:1) and applied to silica gel thin layer plates in a solvent system of benzene-dioxane-acetic acid (60:30:3). The radiochromatogram obtained from the incubation of kidney microsomes and ^{14}C -AA shows radioactive peaks corresponding to PGE_2 , $\text{PGF}_{2\alpha}$, PGD_2 and TXB_2 (thromboxane B_2). Similarly, using another solvent system (chloroform-methanol-acetic acid-water, 90:8:1:0.8) a thromboxane B_2 peak was detected between $\text{PGF}_{2\alpha}$ and PGE_2 . Thus, using two different solvent systems it is possible to demonstrate an arachidonate product which has chromatographic mobility similar to that of thromboxane B_2 . More specific proof of synthesis was obtained by performing the enzymic incubation in the presence of imidazole, a specific antagonist of thromboxane synthetase¹⁴. The thromboxane B_2 peaks were abolished when the renal microsomes were reacted with arachidonate in the presence of 5 mM imidazole (Fig. 3). Similar results, that is, thromboxane B_2 formation which was inhibitable by imidazole, were obtained using ^{14}C -PGH₂ as substrate (instead of ^{14}C -arachidonic acid) with kidney microsomes at 0 °C for 20 min. Only the ureter obstructed kidney seems to possess thromboxane synthetase activity since identical experiments carried out with microsomes prepared from the contralateral unobstructed kidney did not show evidence of a thromboxane B_2 peak on radiochromatograms. These experiments indicate that the microsomal preparation from rabbit kidneys after 3 d of ureteral occlusion can synthesise thromboxane A_2 from arachidonate or the endoperoxide PGH₂. The ureter-obstructed kidney converted 2.5–3% of the total arachidonate radioactivity to

Fig. 2 Bioassay of rabbit aorta constricting substance (RCS). a, Response of the assay strips, rat stomach strip (RSS) (top) and rabbit aortic strip (RA) (lower trace) to PGE_2 160 ng directly over the strips (DIR) and endoperoxide generated from sheep seminal vesicles (SSV) and arachidonate (AA)¹⁸. b, Contractions of RSS and RA to 200 ng bradykinin (BK) through the kidney (TK). This effluent was collected, reoxygenated and reperfused over a second aortic strip, shown in (c). c, Trace showing complete loss of contractile activity on rabbit aorta after 1.5 min but persistence of PGE_2 activity as indicated by contraction of RSS ($n = 10$).

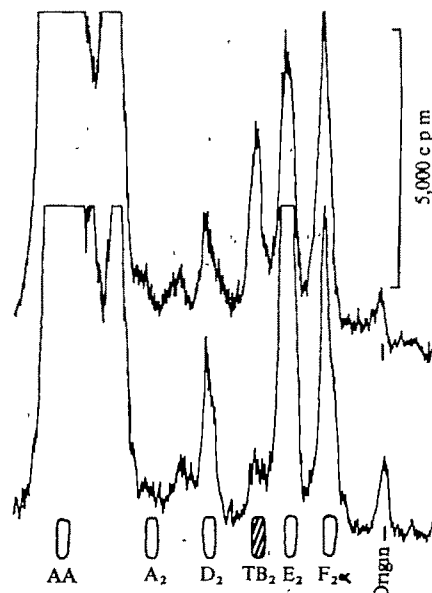
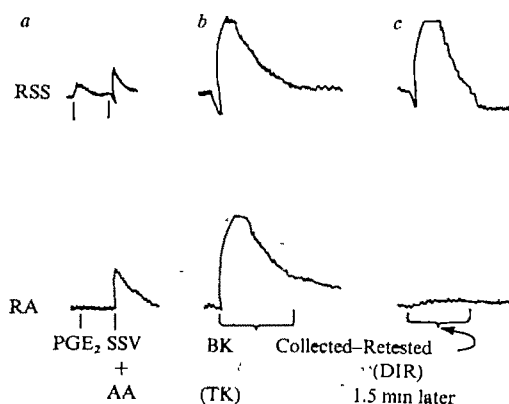


Fig. 3 Thin-layer radiochromatograms of the acid-lipid extract obtained from rabbit kidney microsomes and ^{14}C -arachidonic acid. The upper scan shows the peak of radioactivity between PGE_2 and PGD_2 corresponding to TXB_2 . The lower scan shows loss of this peak when incubations were carried out in the presence of imidazole 5 mM. The mobility of the cold standards in the solvent system of benzene-dioxane-acetic acid (60:30:3) are indicated on the scans. Standards used were $\text{PGF}_{2\alpha}$ ($\text{F}_{2\alpha}$), (E_2) PGE_2 , PGD_2 (D_2), PGA_2 (A_2) and arachidonic acid (AA) (all gifts from Upjohn). TB_2 , thromboxane B_2 ; APM, aspirin treated platelet microsomes ($n = 5$).

^{14}C TXB_2 . The percentage ^{14}C radioactivity converted to PGE_2 , $\text{PGF}_{2\alpha}$ and PGD_2 were 15%, 10% and 5% respectively. Assuming lower rates of conversion for the contralateral kidney, the amount of thromboxane B_2 formed may be below the lower limit of our discrimination.

The unmasking of thromboxane synthetase activity in the experimental model of ureteral obstruction suggests that the biosynthesis of thromboxane A_2 may be important to the alterations in renal vascular resistance and regional blood flow. The potential role of thromboxane A_2 in ureteral obstruction, if it is produced in other species, may be in the alterations in regional blood flow to non-functioning areas of the kidney or decreases in total renal blood flow to a non-functioning kidney in a pathological situation. The capacity to unmask renal thromboxane synthetase activity may give new insight into some old problems.

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Is yawning a cholinergic response?

BARBIZET¹ was perhaps right in suggesting that the commonplace, ubiquitous and apparently non-significant nature of the act of yawning, might have contributed to the relative lack of attention given by physiologists to this behavioural phenomenon. The physiological significance of yawning and the nervous mechanisms triggering and coordinating its various components remain unclear^{1,2}. Frequent yawning has been mentioned as a symptom in some diseases of the central nervous system, particularly in cases of frontal tumours and encephalitis^{3,4}. A stretching and yawning syndrome has been observed in cats, dogs and monkeys after intracisternal or intraventricular injections of adrenocorticotrophic and melanotrophic hormones, or synthetic polypeptides with ACTH and/or MSH activities^{5,6}, but the relation of these observations to the basic mechanism of yawning has not been studied. During experiments designed to explore cholinergic potentiation of D-amphetamine induced head-shaking in infant rats⁷, we have made some observations that indicate the involvement of a central cholinergic response in yawning.

Infant rats, from 6 h after birth up to 14 d old, were injected intraperitoneally (i.p.) with physostigmine salicylate (0.10 mg of the free base per kg body weight, dissolved in 0.9% NaCl to a total volume of 0.01 ml per g bodyweight). The rats began to yawn about 5 min after the injection. In the following 15 min the number of yawns reached an average of 10–12 during the first 10 d of life. Some animals yawned twice per minute, and others only a couple of times in 15 min. In rats aged under 10 d physostigmine induced yawning was practically universal. Between days 10 and 14 of life the frequency of yawning fell rather steeply, to a level of only 2 or 3 yawns per 15 min, with some animals showing no yawning response. At the 14th day yawning occurrence had decreased to 69% of the population. Each yawn was preceded by salivation, licking of the forepaws and cleaning movements of the snout, or 'gumming' or chewing movements, and sometimes by a sudden stretching of the forelimbs. The yawn itself was a slow wide opening of the mouth, with marked protrusion of the tongue, and lasted about 3–4 s (Fig. 1).

A dose-effect graph of physostigmine induced yawning in

Fig. 1 Physostigmine-induced yawning in a male rat (3 months old) injected with physostigmine at 0.10 mg per kg body weight.

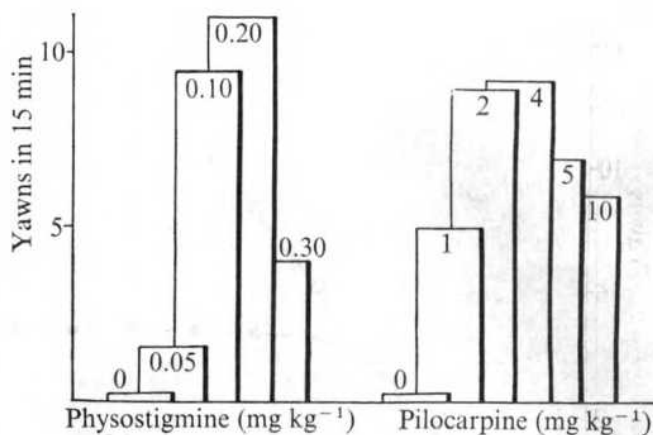


Fig. 2 Physostigmine and pilocarpine induced yawning. Drug doses (free base) are indicated in each column (mg per kg body weight). Each group consisted of 12 nine-day-old rats. All values after drug injection are significantly different from the controls as follows: physostigmine, 0.05 mg, $P < 0.05$; 0.10 and 0.20 mg, $P < 0.001$; 0.30 mg, $P < 0.003$; pilocarpine, all doses tested, $P < 0.001$ (Mann-Whitney U test⁸).

9-d-old rats is shown in Fig. 2. That this particular action of the anticholinesterase drug is centrally mediated is strongly suggested by control experiments with neostigmine methylsulphate, which passes the blood-brain barrier with difficulty. When injected i.p., in a dose of 0.2 mg per kg, neostigmine induced only 1.6 yawns per 15 min (12 animals). Although this effect is significantly different from that observed in rats injected with saline ($P < 0.01$) it was only of the order of one-sixth of the effect obtained with physostigmine. With higher doses of neostigmine no yawning was observed. Pilocarpine hydrochloride, a cholinomimetic drug that passes readily into the brain, induced yawning up to the same level as physostigmine (Fig. 2).

Cholinomimetically induced yawning seems to be due to the stimulation of central 'muscarinic' receptors, because it is rapidly and completely blocked by scopolamine hydrobromide, at doses of 2.5 to 5 mg per kg (Fig. 3). That 'nicotinic' cholinergic receptors are not participating was demonstrated with nicotine administered by i.p. injection. Doses of nicotine (0.1 mg per kg) which induced clear central effects on head-shaking (unpublished observations) did not induce yawning.

We observed physostigmine induced yawning at a more or less stable level of 2–3 yawns per 15 min in rats from 14 to 90 d of age, but practically only in males. In a pool of 62 animals (31 females and 31 males, 14 to 54 d old) from the same litters, males performed an average of 3.1 yawns in 15 min after physostigmine injection, whereas females performed 0.5 yawns in the same period. The difference is highly significant ($P < 0.002$) by the Mann-Whitney U test⁸. It may be recalled that Hall⁹, in his interesting description of the behaviour of monkeys towards mirror-images, in which 'yawning' or 'gaping' responses were very frequent, also observed that adult females 'rarely, if ever, yawned'.

Our results suggest that in the rat there is a central cholinergic mechanism, with 'muscarinic' receptors, underlying the act of yawning. The mechanism matures at a very early age, suggesting that it is localised at the lower levels of the brain stem. Later development of higher placed cholinergic mechanisms may determine that other effects evoked by cholinomimetic agents injected systemically might be hindering the expression of yawning in older rats. Intense and continuous scratching, or tremor and rigidity, as produced respectively by pilocarpine and physostigmine in rats aged over 10 d, or some other central or peripheral

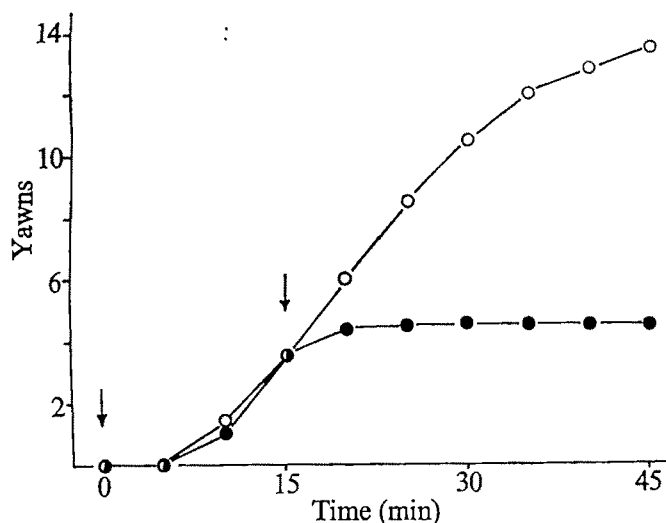


Fig. 3 Blocking effect of scopolamine on physostigmine induced yawning. Arrows indicate the times of injections: at 0 time, physostigmine 0.10 mg per kg; at 15 min, scopolamine 5 mg per kg. The blocking effect of scopolamine is highly significant, $P < 0.001$ already 10 min after injection (Mann-Whitney U test⁶). Similar results were observed in another experimental series with scopolamine, 2.5 mg per kg.

actions affecting the level of vigilance, might be partially counteracting the yawn-inducing effect.

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Myasthenia gravis serum reduces acetylcholine sensitivity in cultured rat myotubes

MYASTHENIA gravis is a neuromuscular disorder characterised by muscle weakness and fatigability. Muscle fibres from myasthenic patients have reduced amplitudes of endplate potentials and miniature endplate potentials^{1,2}. Early studies concluded that the disorder was presynaptic in origin because the postsynaptic chemosensitivity of the myasthenic muscle fibres was found to be normal³, but recent evidence indicates that myasthenia gravis is an autoimmune disease directed against the postsynaptic acetylcholine receptors⁴. Myasthenic serum globulin has been shown to bind to the acetylcholine receptors⁵, myasthenic muscle contains only 11–30% of α -bungarotoxin binding sites compared with normal muscle⁶, and iontophoretic studies showed that the acetylcholine sensitivity of the postsynaptic region of myasthenic muscle was markedly reduced⁷. But the failure of *in vitro* application of myasthenic serum to reduce the junctional or extrajunctional sensitivity of human, rat or frog muscle⁸ has raised doubt on the involvement of the

serum factor in the disease. In this report we show that the acetylcholine sensitivity of cultured rat myotubes is greatly reduced by *in vitro* application of myasthenic serum.

Skeletal muscle cells from newborn rats were explanted on collagen-coated 60 mm plastic Petri dishes according to the techniques of Yaffe⁹, at a plating density of 5×10^4 cells per dish. The cells were incubated in Dulbecco's modified Eagle's medium with 8 g l⁻¹ glucose, 9% foetal calf serum, 0.5% chick embryo extract and 50 μ g ml⁻¹ gentamycin. The cultures were maintained in a humidified atmosphere at 37 °C with 95% air and 5% CO₂. The myogenic cells began to fuse by day three and experiments were performed on 5 to 6 d old myotube cultures. Pooled sera from two patients with myasthenia gravis with a high titre of acetylcholine receptor antibody, and pooled sera from two normal individuals, were used. The normal or myasthenic serum was added to the 5–6 d old cultures containing the culture medium so as to give a sevenfold dilution of the serum.

Intracellular recordings were made using 3 M KCl-filled microelectrodes of 20–40 M Ω resistance, and the acetylcholine sensitivity measured iontophoretically using microelectrodes filled with 2 M acetylcholine and with a resistance of 50–200 M Ω . Recordings were made at 23 °C in the culture media except where otherwise stated. Acetylcholine receptors are located over the entire surface of cultured rat myotubes¹⁰, and the acetylcholine sensitivity was measured at several points on each myotube and the average sensitivity calculated. The sensitivity was expressed in mV nC⁻¹ and corrected for variation in membrane potential by multiplying the observed sensitivity by a factor $(E_s - E_R)/(E_M - E_R)$ where E_s is the standard membrane potential (–60 mV), E_R is the reversal potential for the acetylcholine response (0 mV) and E_M is the resting potential of the cell. The acetylcholine sensitivity was always measured for a depolarisation of 5 mV. It is particularly important to measure the acetylcholine sensitivity using a constant depolarisation when the sensitivity of different cells is being compared, since the sensitivity becomes higher with increasing depolarisation if the dose–response curve is nonlinear. We have found that the action of acetylcholine on rat myotubes does show such cooperativity, with a Hill coefficient of 1.5–2.0.

The acetylcholine sensitivity of the myotubes was initially measured in three separate sets of cultures with half of each set of cultures incubated for 22.5 h in normal serum, and the remainder incubated for the same period in myasthenic serum (Table 1). The myasthenic serum did not alter the resting potential of the myotubes, E_M of the normal and myasthenic cells averaging –47 mV and –45 mV respectively. The acetylcholine sensitivity of the cells treated with myasthenic serum was greatly reduced, however, in all of the three sets of cultures. The average acetylcholine sensitivity of the normal myotubes was 399 mV nC⁻¹, while that of the myasthenic myotubes was 27 mV nC⁻¹. Thus 22.5 h incubation in myasthenic serum caused a 93% reduction in the acetylcholine sensitivity.

Table 1 Reduction of acetylcholine (ACh) sensitivity of cultured rat myotubes by myasthenia gravis serum

Culture	No. of cells	Normal ACh		Myasthenia ACh		Inhibition of ACh sensitivity in myasthenic cells (%)
		E_M (mV)	sensitivity (mV nC ⁻¹)	E_M (mV)	sensitivity (mV nC ⁻¹)	
1	6	32	175	36	12	93*
2	5	50	825	46	52	94*
3	6	55	198	58	16	92*

The myotubes were incubated in normal serum or myasthenic serum for 22 h. Statistical significance was determined using a t test. E_M , membrane potential.

* $P < 0.001$.

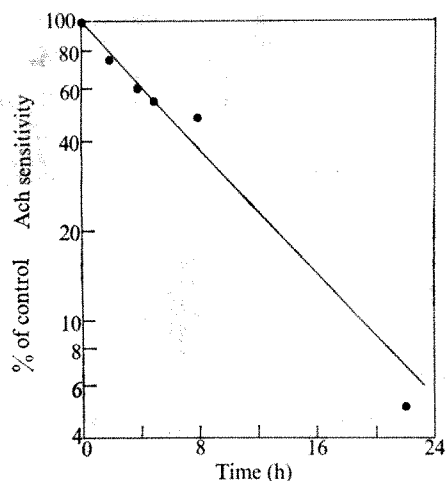


Fig. 1 Acetylcholine sensitivity of cultured rat myotubes incubated for various times in myasthenic serum. Cultures were incubated for the times shown in normal or myasthenic serum, and the average value of the ACh sensitivity of six to eight myotubes cultured in normal or myasthenic serum was compared for each incubation time.

This reduction in sensitivity was irreversible, as perfusing for 3 h in normal saline caused no increase in the acetylcholine sensitivity. The acetylcholine potentials were also much slower in the myotubes treated with myasthenic serum. Normal myotubes gave 5 mV amplitude acetylcholine potentials with 10–20-ms rise times, whereas myotubes incubated with myasthenic serum gave 5 mV potentials with only 50–100-ms rise times. The acetylcholine sensitivity varied slightly at different points on both normal and myasthenic serum treated myotubes, but we found no evidence of acetylcholine receptors accumulating in patches or in caps at one area of the myotubes.

The rate of the decrease in acetylcholine sensitivity was very slow, and 2 h after incubation with the myasthenic serum, the sensitivity had decreased by only 25%. Figure 1 shows the time course of reduction of acetylcholine sensitivity, plotted on a semi-logarithmic scale. The reduction in sensitivity is approximately exponential, with a decay rate constant of 0.124 h^{-1} and a half-time of 5.6 h.

These results demonstrate that the serum of myasthenic gravis patients contains a factor which can reduce the acetylcholine sensitivity of cultured rat myotubes. Previous biochemical studies have shown that this factor is an IgG globulin which can bind to the acetylcholine receptor^{6,8}, although the IgG binding site is different from the acetylcholine binding site^{9,10}.

The slow rate and irreversible nature of the reduction in acetylcholine could be explained by the serum globulin being an irreversible antagonist and present in very low concentrations. The globulin has been shown, however, to produce an acceleration of the internalisation and/or degradation of the surface acetylcholine receptors of the cultured rat myotubes¹¹. It therefore seems likely that the binding of the globulin to the receptor does not prevent activation of the receptor by acetylcholine, but the reduction in acetylcholine sensitivity is caused by a decrease in the density of surface receptors on the myotubes.

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Human myasthenic sera reduce acetylcholine sensitivity of human muscle cells in tissue culture

MYASTHENIA gravis is a muscle disease of man characterised by impaired neuromuscular transmission during repetitive motor nerve activity. Recent studies suggest that the defect in myasthenia gravis is post-synaptic and that myasthenic end-plates have a lower density of functional acetylcholine (ACh) receptors than normal end-plates. Thus end-plates from myasthenic patients show unusually small miniature end-plate potentials^{1,2}, are less sensitive than normal end-plates to iontophoretically applied ACh (refs 2, 3) and bind reduced amounts of α -bungarotoxin^{4,5}—a snake neurotoxin which is considered to bind specifically to nicotinic ACh receptors (see ref. 5). The presence of antibodies against ACh receptors in the sera of myasthenic but not of control patients^{6–10} suggests that anti-receptor antibodies might act in some way to decrease the number of functional receptors at the motor end-plate. Previous investigations, however, have failed to show conclusively that myasthenic sera contain a factor that reduces muscle ACh sensitivity^{3,11–13}. Here we show that myasthenic sera can reduce the ACh sensitivity of tissue cultured human muscle cells.

Explant cultures were made from muscle tissue of 12–16 week old foetuses. Cultures were grown at 37 °C on gelatin-coated tissue culture dishes in a modified Eagle's medium¹⁴ containing 10% foetal calf serum. Outgrowths of fibroblasts and myoblasts were seen after about 2 weeks and fused myotubes appeared after 3 weeks. The cultures selected for study were aged 4–7 weeks.

Iontophoretic application of ACh on to human myotubes evoked depolarising responses (Fig. 1). To compare ACh responses in cells of different resting potentials, ACh sensitivities were corrected to a resting potential of -50 mV by multiplying the recorded sensitivities by $-50/RP$, where RP is the resting potential (mV) of the cell under study. This correction factor assumes that the reversal potential for ACh responses is 0 mV (our unpublished observations). Cultures pre-incubated with 10% normal human serum at 37 °C for 1.5 h were used to provide a control value for ACh sensitivity. The mean sensitivity of myotubes exposed to three different normal sera was $1,080 \text{ mV nC}^{-1}$ (range of mean values, 800–1,200 mV nC^{-1}), which is similar to that of untreated cultures (about 950 mV nC^{-1}).

Six myasthenic sera were tested for their effect on myotube ACh sensitivity. Cultures were incubated with 10% serum at 37 °C for 1–1.5 h before electrophysiological examinations in serum free solution. As shown in Table 1, all the sera reduced myotube ACh sensitivity; in some cases the reduction was surprisingly large. This lowering of sensitivity is complement independent since a similar decrease was seen for one serum (no. 6 in Table 1) when complement was inactivated by heating the serum to 56 °C for 30 min. Myotube electrical properties which can influence the measured ACh sensitivity were similar in cultures exposed to either normal or myasthenic sera (Table 2). The lower sensitivity of myotubes treated with myasthenic sera must, therefore, result from an action of the serum on ACh receptors. Two sera from patients with

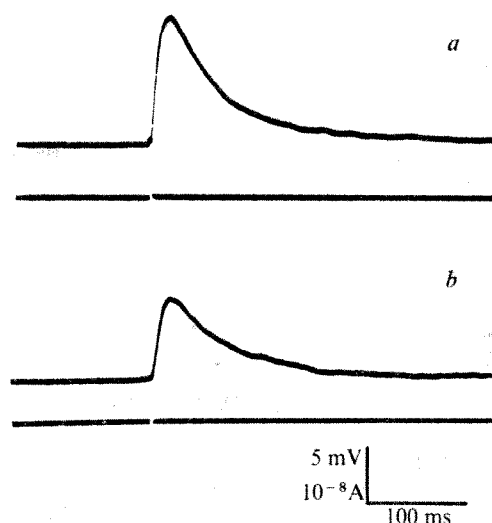


Fig. 1 Depolarising responses evoked by iontophoretic ACh pulses of 1 ms duration in myotubes treated with *a*, normal human serum and *b*, myasthenic human serum (serum 2 in Table 1). Recordings were made at 23 °C in a serum-free solution containing (mM) NaCl 150, KCl 5, CaCl₂ 10, HEPES-NaOH 5, pH 7.4. ACh was applied iontophoretically from pipettes filled with 3 M AChCl (resistances 180–400 MΩ using pulses of 5–20 nA amplitude and 0.5–10 ms duration). The position of the ACh pipette and the negative backing current required to prevent ACh diffusing out of the pipette were adjusted for each myotube to ensure a maximal ACh response. The resultant myotube depolarisations were recorded with an intracellular microelectrode filled with 4 M potassium acetate (resistances 20–40 MΩ). Recording pipettes were bevelled²⁷ to facilitate penetration of the small myotubes. ACh sensitivities were calculated as mV depolarisation per nanocoulomb (nC) of charge passed through the iontophoretic pipette.

Duchenne muscular dystrophy and amyotrophic lateral sclerosis were also tested; these had little or no effect on ACh sensitivity.

An immunoglobulin (IgG) fraction of myasthenic sera has been shown to block α -bungarotoxin binding to ACh receptors^{6,7}. To test if IgG is also involved in reducing ACh sensitivity, one serum was treated with goat anti-human IgG to precipitate IgG. The IgG-depleted serum showed a greatly reduced activity whereas serum treated with normal goat serum retained its full potency (see Table 1).

The effect of incubating cultures with myasthenic sera at a lower temperature was tested in four cases. Cultures were incubated with 10% serum for 1.5 h at 23 °C before estimating ACh sensitivities. All four sera reduced myotube sensitivity, though the decrease in each case was less than when cultures were exposed to myasthenic serum at 37 °C (Table 1). In two of these experiments, the cultures were subsequently incubated in serum-free medium at 37 °C for an additional 1 h. This resulted in a further decrease in sensitivity; the relative sensitivity of cells incubated with sera 3 and 6 fell from 0.41 and 0.47, respectively, to 0.18 in both cases. From this result we conclude that the temperature dependence of the serum effect is not due to different amounts of antibody binding at the two temperatures, but rather reflects a subsequent step in antibody action.

Experimentally raised anti-receptor antibodies increase the rate of receptor degradation in cultured rodent muscle cells¹⁵. This degradation, which resembles antigenic modulation^{16,17}, is temperature dependent and is blocked by metabolic inhibitors. The similar temperature dependence of human myasthenic serum action suggests that antibodies in the sera act to raise the receptor degradation rate in human myotubes. Indeed when five of the myasthenic sera were tested on cultured mouse muscle cells, all were found to increase the rate of ACh receptor degradation (Fig. 2). Receptor degradation follows first-order kinetics^{18–21},

Table 1 Effect of human sera on the ACh sensitivity of human myotubes

Serum	Anti-human ACh receptor titre ($\times 10^{-9}$ M)	Relative ACh sensitivity	
		23 °C	37 °C
Normal	<0.6	1.00	1.00
Myasthenic patient no.			
1	30	0.46	0.02
2	8	—	0.39
3	446	0.69, 0.41	0.20
4	332	0.71	0.40
5	143	—	0.53
6	83	0.47	0.03
6, complement inactivated	—	—	0.06
6, IgG precipitated with goat anti-human IgG	—	—	0.65
6, plus normal goat serum	—	—	0.08
Duchenne muscular dystrophy	<0.6	—	0.95
Amyotrophic lateral sclerosis	<0.6	—	1.19

Relative ACh sensitivities of myotubes after exposure to 10% human sera at either 23 or 37 °C for 1–1.5 h. Sensitivities are expressed as the ratio (test serum ACh sensitivity–normal serum ACh sensitivity). For each serum sensitivities were estimated in 6–20 cells. The unity value for normal serum represents the mean sensitivity of six experiments testing three sera. Anti-human ACh receptor titres were estimated using an immunoprecipitation technique described elsewhere²⁸. Titres are expressed as moles of ¹²⁵I- α -bungarotoxin bound to human ACh receptors precipitated per litre of serum. In one experiment, serum 6 was depleted of IgG by precipitation with goat anti-human IgG serum; complement was inactivated in both sera. The resultant supernatant was dialysed against culture medium for 24 h before adjusting the volume to give the equivalent of 10% human serum in medium. Some reduction in sensitivity was seen when this IgG-depleted serum was tested. It seems likely that this is due, at least in part, to the lower input impedances of the myotubes in this experiment (about 40% of other values). To test for any general effects of goat serum on myasthenic serum action, the immunoprecipitation protocol was repeated using normal goat serum in place of immune goat serum. In this case no precipitate was formed and the full reduction in ACh sensitivity was seen.

whereas the appearance of new receptors is a linear process^{19,21,22}. An antibody-induced acceleration of receptor degradation without a compensatory increase in the rate of synthesis would therefore result in a lowered receptor density; this could explain the reduced ACh sensitivity of cells exposed to myasthenic sera. A possible objection to this idea is the observation that while the ACh sensitivity of myasthenic serum-treated cells declines within 60–90 min, the accelerated secretion of degraded ¹²⁵I- α -bungarotoxin, which is used to assay receptor degradation, occurs after a lag of several hours (Fig. 2 and ref. 15). This lag, however, includes the time not only for induced receptor internalisation but also for receptor catabolism and secretion of the degraded toxin. The internalisation of antibody-complexed cell surface components can occur within 1 h (ref. 23) and the secretion of degraded ¹²⁵I- α -bungarotoxin occurs in

Table 2 Myotube electrical properties after exposure to human serum at 37 °C

	Resting potential (mV)	Input impedance (MΩ)	Membrane time constant (ms)
Normal sera	-47.9 $\pm$ 4.4	244 $\pm$ 76	71 $\pm$ 17
Myasthenic sera	-47.7 $\pm$ 3.7	225 $\pm$ 28	77 $\pm$ 8

Values are expressed as mean $\pm$ s.e. of between experiment results. The measured resting potentials represent the potentials at which ACh sensitivities were measured and probably underestimate the true value. Cell input impedances and membrane time constants were measured by passing hyperpolarising pulses of 0.2 nA amplitude and up to 1 s duration through the recording electrode using a bridge circuit built into the electrometer (M-4A, W.P. Instruments). As the time constant of the myotube membrane was much greater than that of the recording system, the bridge circuit was easily balanced with the electrode inside the cell. The large input impedances reflect the small size of the cells (5–20 μ m diameter) rather than any unique membrane properties.

normal conditions with a lag of about 1 h (ref. 19). An antibody-induced modulation of ACh receptors may, therefore, lower the surface receptor density within the 1–1.5 h incubation periods used in the electrophysiological experiments.

Bound antibodies could also reduce ACh sensitivity by modifying the properties of activated ACh receptors. Preliminary 'ACh noise' experiments indicate that in human myotubes exposed to myasthenic serum the charge passed during a single channel opening is reduced by about 30% (S.B., R.W.K. & J. Rice, unpublished). A similar reduction in the charge transferred per open channel is seen in cultured rat myotubes treated with experimentally raised antibodies¹⁵. In addition, antibodies might act by completely inactivating some ACh receptors. Such direct actions of antibodies on receptor function may explain the lowered ACh sensitivity of cells exposed to myasthenic serum at 23 °C and also contribute to the larger reduction seen with incubations at 37 °C.

Our demonstration that myasthenic sera can reduce muscle ACh sensitivity contrasts with the results of earlier studies. As anti-receptor antibodies show a higher affinity for homologous than for heterologous receptors^{24,25} the lack of any serum effect in some previous studies may be due to the use of non-human muscle cells. In addition, more labile receptors such as those in tissue cultured cells^{19,21} may be necessary to show a rapid reduction in sensitivity arising

from receptor modulation. The loss of the more stable end-plate receptors^{18,20,26} through modulation may occur too slowly to cause any decrease in ACh sensitivity during short term experiments. This would explain the apparent absence of any blocking action of myasthenic sera at human end-plates *in vitro*³.

Our results suggest that the low ACh sensitivity of human myasthenic end-plates results primarily from the action of antibodies. Further experiments will be necessary to determine if myasthenic sera do decrease human end-plate sensitivity in appropriate conditions and to elucidate further the importance of receptor modulation in depressing ACh sensitivity.

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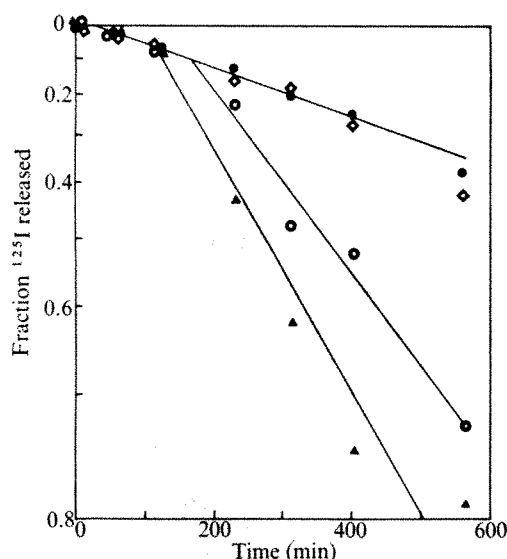


Fig. 2 Loss of ¹²⁵I-α-bungarotoxin from mouse muscle cells. Cells of the clonal cell line BC₃H-1 (refs 15, 21) were incubated in 10⁻⁸ M ¹²⁵I-α-bungarotoxin for 2 h at 21–23 °C in medium containing 0.2% foetal calf serum. Unbound toxin was then removed by washing the cells four times in medium with 0.2% foetal calf serum. The cultures were then incubated at 37 °C in medium containing various sera and the amount of ¹²⁵I released into the medium measured at various times. ¹²⁵I release is expressed as the ratio ¹²⁵I released/amount of ¹²⁵I initially bound to cells. Each point is the mean of duplicate samples. Fractionation of the medium on a Biogel P2 column showed that the extra ¹²⁵I released in the presence of myasthenic serum is due to an increase in the release of low molecular weight ¹²⁵I-labelled material and not to an induced dissociation of intact bungarotoxin. ◇, Foetal calf serum (FCS, 12%); ●, 10% normal human serum + 2% FCS; ○, 10% myasthenic human serum + 2% FCS. For comparison the effect on these cells of serum from rats immunised with electric eel ACh receptor is also shown (▲, 10% rat serum + 2% FCS, final titre 1.1 × 10⁻⁸ M anti-rat receptor). In a further experiment 10% human serum was added to cultures in the presence of 10⁻⁸ M ¹²⁵I-α-bungarotoxin and 10⁻³ M curare. In these conditions toxin binding to the muscle cells is inhibited by more than 90%. After 600 min the medium was removed and fractionated. No degradation of the ¹²⁵I-α-bungarotoxin was noted, indicating that the myasthenic serum, by itself, does not degrade toxin and that toxin degradation only occurs after binding to ACh receptors.

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Long term β-adrenergic blockade reduces tyrosine hydroxylase and dopamine β-hydroxylase activities in sympathetic ganglia

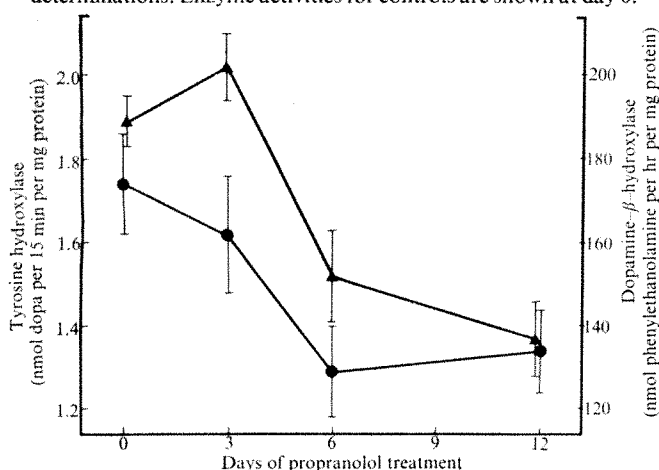
β-ADRENOCEPTOR antagonists are now well established in the treatment of hypertension but the mechanism by which they reduce blood pressure is not understood¹. There is clearly more involved than an immediate blockade of the cardiovascular β-adrenergic receptors, since intravenous (i.v.) infusion of propranolol in man reduces cardiac output but has no effect on arterial blood pressure^{2,3}. The full hypotensive effect of β-adrenergic blockers is delayed⁴, and is associated with a reduction in peripheral resistance^{2,3}. The hypotensive action of these drugs is nevertheless predominantly related to their common property of β-adrenergic receptor blockade, as they all produce similar

falls in blood pressure⁵ despite their differing ancillary properties⁶ and effects on plasma renin activity⁷. It has been suggested that the hypotensive effect of propranolol is due to a central inhibition of sympathetic activity, since i.v. propranolol reduces preganglionic sympathetic nerve activity in rabbits⁸. But practolol, an effective antihypertensive which penetrates the central nervous system poorly⁹, has no such acute effect¹⁰. The possibility has yet to be considered that the hypotensive action common to all β -adrenoceptor antagonists is directly related to a slowly developing reduction in sympathetic activity and hence in peripheral resistance. Long term increases or decreases in the activity of sympathetic neurons are specifically reflected by changes in the concentration of the nor-adrenaline-synthesising enzymes tyrosine hydroxylase and dopamine β -hydroxylase contained in the cells¹¹. We report here that prolonged treatment with β -adrenoceptor antagonists causes a slow-onset reduction in the concentration of these enzymes in the superior cervical ganglia of rabbits. This decrease in sympathetic activity may in part explain the hypotensive effect of long-term β -blockade.

Rabbits (2–3 kg) were injected twice daily subcutaneously (s.c.) with ($\pm$)-propranolol for 3, 6 or 12 d, or with saline. The degree and duration of β -blockade achieved with this dosage regime have been previously reported¹². In further experiments animals were dosed for 24 d with either ($\pm$)-propranolol, (+)-propranolol (ICI), ($\pm$)-metoprolol, ($\pm$)-acebutolol, ($\pm$)-practolol in equipotent dosage or with saline. Animals were stunned 18–24 h after the final injection and the left and right superior cervical ganglia were rapidly removed and homogenised separately in 1 ml of 0.3 M sucrose. Tyrosine hydroxylase activity of homogenates was measured by the method of Levitt *et al.*¹³ and dopamine β -hydroxylase activity by that of Molinoff *et al.*¹⁴. Protein was determined by the method of Lowry *et al.*¹⁵. To compare the effect of decentralisation on ganglionic enzyme activity with that of prolonged β -adrenoceptor blockade, the left superior cervical preganglionic trunk was cut and ligated under nembutal anaesthesia in four rabbits. After a recovery period of 12 d both superior cervical ganglia were removed and the activities of their enzymes were measured.

Propranolol has no acute effect on tyrosine hydroxylase activity in sympathetic ganglia¹⁶ and similarly we found no significant change in ganglionic tyrosine hydroxylase or dopamine β -hydroxylase activities after 3 d of propranolol treatment. In contrast, activities of both enzymes were reduced by pretreatment with propranolol for 6 d (Fig. 1),

Fig. 1 Effect of chronic treatment with ($\pm$)-propranolol on activities of tyrosine hydroxylase (●) and dopamine β -hydroxylase (▲) in rabbit superior cervical ganglia. Rabbits were injected s.c. twice daily with ($\pm$)-propranolol 4 mg per kg body weight for 3 d ($n = 4$), 6 d ($n = 4$) or 12 d ($n = 4$) or with saline 1 ml per kg ($n = 6$). Points are mean $\pm$ s.e.m. of 8–12 determinations. Enzyme activities for controls are shown at day 0.



and after 12 d tyrosine hydroxylase and dopamine β -hydroxylase activities were 25% lower than in controls. To determine whether this action was due to β -receptor blockade and not to any subsidiary properties of the drugs, rabbits were treated for 24 d with four β -adrenoceptor antagonists chosen for their differing properties of cardio-selectivity, intrinsic sympathomimetic activity and membrane-stabilising action⁶. Despite these differences all four drugs produced comparable reductions in enzyme activities (Table 1). The fall in dopamine β -hydroxylase activity was similar for all drugs, but the reduction in tyrosine hydroxylase activity was greatest with propranolol, and least with practolol. This cannot be due to the relatively powerful membrane-stabilising activity of propranolol since (+)-propranolol, which has negligible β -blocking activity but the same local anaesthetic potency as ($\pm$)-propranolol, did not alter enzyme activities (Table 1). The difference between propranolol and practolol may be related to the high lipid solubility and central nervous system (CNS) penetration of propranolol¹⁷ compared with that of practolol⁸.

Table 1 Effect of chronic treatment with β -adrenoceptor antagonists on tyrosine hydroxylase and dopamine β -hydroxylase activities in rabbit superior cervical ganglia

	Tyrosine hydroxylase (% of control)	Dopamine β -hydroxylase (% of control)
($\pm$)-Propranolol	64 $\pm$ 8*	70 $\pm$ 6*
(+)-Propranolol	91 $\pm$ 10	103 $\pm$ 8
($\pm$)-Metoprolol	70 $\pm$ 7*	73 $\pm$ 6*
($\pm$)-Acebutolol	79 $\pm$ 7†	64 $\pm$ 13†
($\pm$)-Practolol	85 $\pm$ 5‡	73 $\pm$ 5*

Rabbits were injected s.c. twice daily for 24 d with either saline 1 ml per kg ($\pm$)-propranolol 4 mg per kg, (+)-propranolol 4 mg per kg, ($\pm$)-metoprolol 6 mg per kg, ($\pm$)-acebutolol 10 mg per kg or ($\pm$)-practolol 10 mg per kg body weight. Enzyme activity is expressed in terms of nmol dopa per 15 min per mg protein (tyrosine hydroxylase) or nmol phenylethanolamine per h per mg protein (dopamine β -hydroxylase) given as percentage of control activity. Values are mean $\pm$ s.e.m. of 10–12 determinations.

* $P < 0.001$.

† $P < 0.02$.

‡ $P < 0.05$.

The effect of decentralisation of superior cervical ganglia is shown in Table 2. Twelve days after decentralisation, activities of tyrosine hydroxylase and dopamine β -hydroxylase were 26% and 29% less than in contralateral control ganglia, a reduction comparable with that caused by prolonged β -adrenergic blockade.

Adaptation to increases in sympathetic activity by trans-synaptic induction of tyrosine hydroxylase and dopamine β -hydroxylase is due to increased synthesis of enzyme protein¹¹. This process is slow, and first detectable 18 h after increased preganglionic activity¹⁸. Conversely it is thus possible that β -adrenoceptor antagonists cause a sudden reduction in preganglionic nerve activity, which is detected in these experiments as a reduced enzyme activity in the sympathetic neurons. As we observed no significant change in enzyme activities after 3 d of propranolol treatment, however, it is more probable that continuing β -adrenergic blockade causes a gradual reduction in preganglionic sympathetic activity mirrored by the reduction in the activities of the specific neuronal enzymes. Strikingly, we found that interruption of preganglionic impulse flow by decentralisation resulted in a very similar fall in the enzyme activities in the superior cervical ganglia. A possible interpretation of this is that chronic β -adrenergic blockade is virtually abolishing all preganglionic nervous activity. Of course, we cannot know if this is so because it is not known by how much the nervous activity must be reduced to detect the consequent decrease in enzymic activity. If preganglionic

Table 2 Effect of decentralisation on the tyrosine hydroxylase and dopamine β -hydroxylase activities in rabbit superior cervical ganglia

	Tyrosine hydroxylase (nmol dopa per 15 min per mg protein)	Dopamine β -hydroxylase (nmol phenylethanol- amine per h per mg protein)
Control (R)	2.02 $\pm$ 0.25	209 $\pm$ 9
Decentralised (L)	1.47 $\pm$ 0.18*	149 $\pm$ 16*

Left superior cervical ganglia were decentralised in four rabbits, and 12 d later the activities of the enzymes were determined. Values given are the mean $\pm$ s.e.m. of four ganglia. To ensure that the ganglia were decentralised choline acetyltransferase activity was determined in the homogenates according to the method of Fonnum²⁰. The activity of this enzyme in the decentralised ganglia was less than 10% of the contralateral controls.

* $P < 0.01$ (paired t test).

activity is reduced by blockade of central β -adrenoceptors* our results would suggest that polar 'cardioselective' drugs may reach significant concentrations in the CNS during steady-state dosing. Alternatively, the reduction in sympathetic activity by chronic β -adrenergic blockade may be peripherally mediated either through a long term alteration of afferent autonomic input, resulting in an adaptive reduction in sympathetic outflow^{4,10}, or through continued blockade of a prejunctional β_1 -adrenoceptor, activation of which increases noradrenaline release¹⁹.

It seems reasonable, though, to conclude that prolonged β -adrenergic blockade produces a slow-onset reduction in the activity of the sympathetic nervous system. Since tyrosine hydroxylase is the rate-limiting enzyme in the biosynthesis of noradrenaline²¹, the amount of neurotransmitter available for release is most probably also reduced. Significant reductions in the ganglionic activities of tyrosine hydroxylase and dopamine β -hydroxylase were seen with several different β -adrenoceptor antagonists, all of which are clinically effective antihypertensives despite their differing subsidiary properties. We therefore suggest that this delayed reduction in sympathetic activity contributes to the hypotensive action of β -adrenoceptor antagonists.

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β Endorphin inhibits ACh turnover in nuclei of rat brain

NARCOTIC drugs modify cholinergic mechanisms in central and peripheral nervous systems¹, and analgesic doses of morphine, meperidine, viminal R₂ and azidomorphine decrease acetylcholine (ACh) turnover rate (TR_{ACh}) in selected brain nuclei^{2,3}. This suggests that endogenous opiate receptor agonists regulate cholinergic mechanisms in some brain nuclei². Among the endogenous opiate receptor agonists, β endorphin is particularly interesting because it is more active than morphine in causing analgesia and catalepsy⁴. This polypeptide and α endorphin share some structural similarities which correspond to fragments 61-76 and 61-91 of β lipotropin, respectively⁵. We have investigated whether α and β endorphin also mimic the action of morphine on TR_{ACh} of selected brain nuclei. We have found that analgesic doses of β endorphin injected intraventricularly decrease TR_{ACh} in cortex, hippocampus, nucleus accumbens and globus pallidus but not in nucleus caudatus. This decrease of TR_{ACh} was antagonised by naltrexone. In contrast, α endorphin failed to cause analgesia or to decrease TR_{ACh} in all brain nuclei studied.

Endorphins were injected into unanaesthetised rats (150 g male Sprague-Dawley, Zivic Miller, Allison Park, Pennsylvania) through polyethylene cannulae permanently implanted in the lateral ventricles, 2 d before the experiments. Each polypeptide was dissolved immediately before use in 10 μ l of saline. The analgesic activity of α and β endorphins was measured by the tail flick method⁷ 30 min after intraventricular injection.

TR_{ACh} was measured by intravenous infusion of phosphorylcholine (ammonium salt) (15 μ mol min⁻¹) for 9 min beginning 21 min after injection of endorphins. During the administration of phosphorylcholine rats were restrained in plastic cages. TR_{ACh} was calculated from the deuterium enrichment of choline (Ch) and ACh as before⁸. Rats were killed with a high-intensity microwave beam focused on the head⁹. Brains were removed and sliced into 400- μ m thick coronal sections and punches were made in the head of the nucleus caudatus, in the pars dorsalis of the hippocampus, in the globus pallidus, nucleus accumbens and parieto-occipital cortex. The position of the polyethylene cannulae was controlled during slicing.

The enrichment in deuterium of ACh and Ch extracted from various nuclei was measured mass fragmentographically⁸. From these values we calculated the fractional rate constant for the efflux of deuterated Ch and ACh. This value multiplied by the ACh content of each brain region gave TR_{ACh} (ref. 10). Table 1 shows that β endorphin inhibited TR_{ACh} in the hippocampus, nucleus accumbens, globus pallidus and cortex. But, like morphine, this polypeptide failed to change TR_{ACh} in the nucleus caudatus. From the data of Table 2, the dose of β endorphin that, given intraventricularly, reduced TR_{ACh} in hippocampus by 50%, was calculated to be approximately 0.2 nmol. From previous reports^{2,3}, the dose of morphine that caused similar changes in TR_{ACh} in the hippocampus was about 33 nmol g⁻¹, given intraperitoneally. Even allowing for differences due to the different route of drug administration, there is no doubt that β endorphin is several times more active than morphine in decreasing the TR_{ACh} of the hippocampus. Using the tail flick test, we have confirmed⁴ that β endorphin has analgesic activity (Table 2): 1.8 nmol per brain caused 50% of maximal analgesia⁷. Similar analgesic activity was obtained with morphine (35 nmol g⁻¹)². Thus, as shown for morphine, doses of β endorphin which elicited analgesia also reduced hippocampal TR_{ACh}. Naltrexone (14 μ mol kg⁻¹, given intraperitoneally) completely prevented the analgesic action and

Table 1 Effects of α and β endorphins on TR_{ACD} in nuclei of rat brain

Brain nuclei	Saline	β Endorphin (2.9 nmol per brain)	β Endorphin + naltrexone	α Endorphin (11.5 nmol per brain)
Cortex	1.0 $\pm$ 0.10	0.35 $\pm$ 0.059*	1.1 $\pm$ 0.14	0.97 $\pm$ 0.14
Hippocampus	3.9 $\pm$ 0.70	0.65 $\pm$ 0.099*	4.9 $\pm$ 2.0	3.7 $\pm$ 0.64
Globus pallidus	2.6 $\pm$ 0.40	1.0 $\pm$ 0.18*	2.9 $\pm$ 0.65	2.13 $\pm$ 0.19
Nucleus accumbens	4.9 $\pm$ 1.0	2.02 $\pm$ 0.35*	4.7 $\pm$ 1.0	4.1 $\pm$ 0.47
Nucleus caudatus	8.6 $\pm$ 0.80	8.4 $\pm$ 1.6	9.9 $\pm$ 1.6	6.7 $\pm$ 2.0

Data expressed in nmol per mg protein per h.

Endorphins were injected intraventricularly 21 min before intravenous infusion of phosphoryl (d_6) choline; naltrexone (14 μ mol per kg) was injected intraperitoneally 15 min before the endorphins. The ACh content in nmol per mg protein was 0.16 $\pm$ 0.03 in cortex, 0.30 $\pm$ 0.020 in hippocampus, 0.73 $\pm$ 0.060 in nucleus caudatus, 0.45 $\pm$ 0.034 in globus pallidus and 0.79 $\pm$ 0.045 in nucleus accumbens; the Ch content was 0.35 $\pm$ 0.030 in cortex, 0.33 $\pm$ 0.032 in hippocampus, 0.28 $\pm$ 0.020 in nucleus caudatus, 0.30 $\pm$ 0.020 in globus pallidus and 0.50 $\pm$ 0.035 in nucleus accumbens. The endorphins and naltrexone failed to change ACh and Ch content. Each value is the mean $\pm$ s.e.m. of 10 assays in the saline-treated group, and of five assays in the other groups.

* $P < 0.01$ compared with the saline-treated group.

the decrease of TR_{ACh} elicited by β endorphin. α Endorphin, in a dose of 11.5 nmol per brain, had no analgesic activity and failed to change TR_{ACh} in any of the five brain areas studied (Table 1). Thus β endorphin was biochemically and behaviourally more potent than α endorphin. This finding is in line with other reports showing the same order of potency in the displacement of ^3H -etorphine from synaptosomal preparations of rat brain¹¹ or in behavioural effects⁴.

Table 2 Analgesia and TR_{ACh} in rat hippocampus after various doses of β endorphin

Dose of β endorphin (nmol per brain)	TR_{ACh} (nmol per mg protein per h)	Analgesia (% of maximum)
0 (saline)	4.1 $\pm$ 0.90	0
0.029	3.8 $\pm$ 0.70	7 $\pm$ 1
0.29	1.0 $\pm$ 0.15*	23 $\pm$ 5
1.45	—	36 $\pm$ 5
2.9	0.65 $\pm$ 0.099*	75 $\pm$ 15

Each value represents mean $\pm$ s.e.m. of 10 assays in saline-treated rats and five assays in endorphin-treated rats. Analgesia was evaluated by the tail flick test⁷.

* $P < 0.01$.

β Endorphin is an active, naturally occurring opiate receptor agonist, which, like morphine, decreases TR_{ACh} in doses which increase the threshold for pain sensation. The data reported here imply that if endogenous β endorphin were to be released from presynaptic terminals in the vicinity of opiate receptors located in the membrane of cholinergic neurones, it would decrease the metabolic rate of ACh and presumably decrease the rate of firing of these neurones.

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Calcium-dependent presynaptic action of substance P at the frog neuromuscular junction

SUBSTANCE P, an 11 amino acid peptide found in many areas of the nervous system¹, has been proposed to be the excitatory transmitter between primary afferents and motoneurons². A depolarising action of substance P on spinal motoneurons and primary afferents has been demonstrated^{3–6}. But, an initial reduction of response preceding an increased spinal reflex discharge and a reduction of the motoneurone excitatory postsynaptic potential has also been seen in response to substance P (refs 4, 5). In addition, both excitatory and inhibitory effects of substance P on firing of cuneate and cortical Betz cells have been reported^{7,8}. In the hatchetfish Mauthner fibre–giant fibre synapse, substance P has a pronounced presynaptic effect⁹. We have further investigated the effects of substance P at a synapse in which the electrophysiological characteristics and transmitter are known, the ionic constituents of the Ringer can be manipulated and quantal analysis is possible—the neuromuscular junction of the frog. We report here that substance P has a potent presynaptic effect on transmission at this synapse which is composed of an initial phase in which transmission is depressed, followed by a prolonged period in which excitability and quantal release are increased.

The cutaneous pectoris muscle and nerve of the frog, *Rana pipiens*, was used. With the exception of experiments (noted below) in which the Ca^{2+} concentration was varied, the Ringer contained 115 mM NaCl, 2 mM KCl, 0.6 mM CaCl and 5 mM MgCl buffered to pH 7.3 with Tes (Sigma). Substance P (Bachem) or eleodisin (eleodisin related peptide, Sigma) was added to the control Ringer, bath applied, and removed with repeated washing after 2–5 min exposure to the preparation. Substance P was used in the majority of the experiments; however, no difference in experimental results was seen when equivalent concentrations of eleodisin were used. Spontaneous miniature endplate potentials (m.e.p.p.) used for frequency and amplitude analysis were recorded from different experiments than those using evoked endplate potentials (e.p.ps). The e.p.ps were evoked by nerve stimulation at 1 per s for 1 min and stimulation discontinued between sampling periods. The m.e.p.ps used for quantal analysis were recorded before the evoked re-

sponse. Quantal content was calculated as e.p.p./m.e.p.p. amplitude. Control experiments of equal duration and stimulation pattern were run. Data analysis was as detailed previously⁹.

The effects of substance P on synaptic transmission were dose dependent and at high doses only depressant effects were seen. At 10^{-4} M concentration, m.e.p.p. amplitude and frequency, e.p.p. amplitude and quantal content were all reduced with the maximum depression being reached at 5–10 min after application. The m.e.p.p. amplitude usually showed a complete recovery but m.e.p.p. frequency, e.p.p. amplitude and quantal content seldom recovered to control values even 1–2 h after application. At lower peptide concentrations (10^{-5} M, 10^{-6} M), recovery of synaptic depression and a second phase of increased synaptic efficacy was seen. An initial depression of synaptic transmission was seen with a maximum depression at 5–10 min and a return to control values after 30–40 min followed by a prolonged second phase of increased synaptic efficacy in which the e.p.p. amplitude and quantal content showed increases of up to five times control values. This phase did not decline to control values until 2–3 h or more after peptide application. The m.e.p.p. amplitude showed little or no change at the lower peptide concentrations. The m.e.p.p. frequency changes usually showed a small frequency reduction in the initial phase and either no change or a small frequency reduction in the second phase. The latency of onset of the peptide effect was approximately 30 s at all peptide concentrations. No change in the resting potential of the muscle fibre was seen at any peptide concentration.

Figure 1 illustrates a typical response to application of 10^{-5} M substance P bath applied at the first arrow and removed at the second arrow. Both e.p.p. amplitude and quantal content reach a maximum reduction by 10 min,

Fig. 1 Representative example of the effect of substance P (10^{-5} M) on *a*, e.p.p. amplitude and *b*, quantal content. Substance P Ringer is applied to the bath at the first arrow and removed at the second. Data plotted as means and standard deviations at each indicated time period.

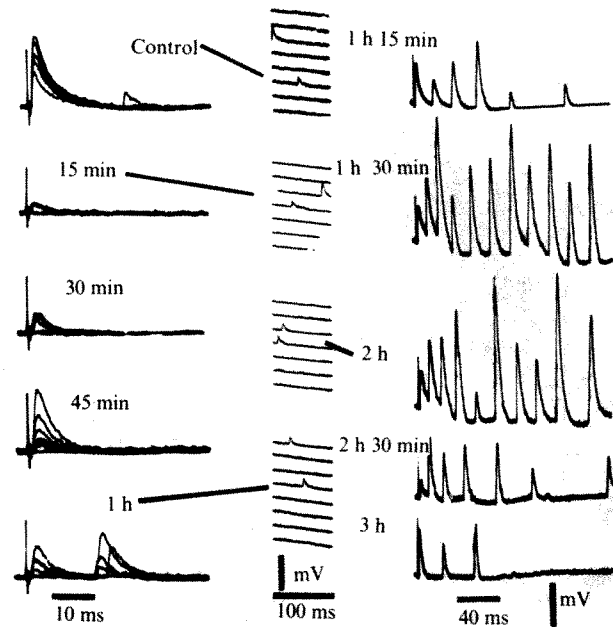
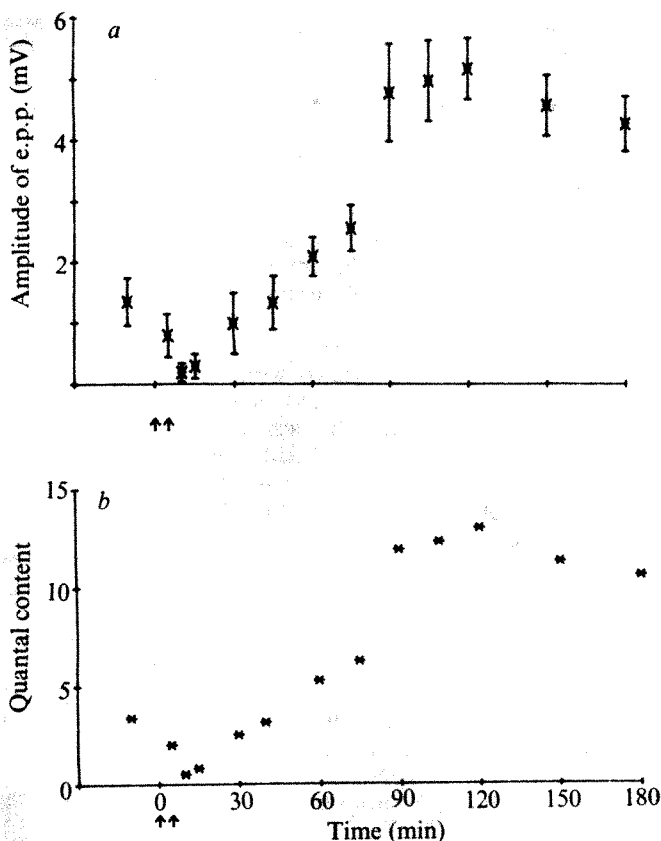


Fig. 2 Multiple e.p.ps recorded from a muscle fibre following substance P application. Substance P (10^{-5} M) is applied after control record and removed by washing 5 min later. The time course of ensuing synaptic depression and recovery is the same as in Fig. 1 but on recovery, multiple e.p.ps are produced in response to a single stimulus to the motor nerve. Figures in first column are composed of five consecutive sweeps; those in the last column are single sweeps. Note difference in time scale for first and last columns. Middle column contains samples from the same fibre of m.e.p.ps at different time periods to illustrate lack of peptide effect on e.p.p. amplitude.

recover to control values at 40 min and then show a prolonged period of increase which, at 3 h post application when the cell was lost, was declining but had not yet returned to control values. In some experiments, during the phase of increased synaptic efficacy, multiple e.p.ps were produced in response to a single stimulus to the presynaptic axon. This is presumed to be due to multiple firing of the motor axon. In Fig. 2, a maximum of 11 e.p.ps are seen in response to a single stimulus to the motor axon. The multiple firing begins as the e.p.p. amplitude returns to control values and lasted, in this case, until the cell was lost 3 h after peptide application. The m.e.p.p. amplitude (middle column) shows no change during the entire experimental period. Although not apparent from the small m.e.p.p. samples in these records, m.e.p.p. frequency showed only a small reduction in frequency after peptide application, lasting for the duration of the experiment.

The initial phase of peptide-induced synaptic depression seems to be calcium dependent (Fig. 3). The reduction in e.p.p. amplitude produced by 10^{-4} M substance P can be blocked by increasing the Ca^{2+} in the Ringer. When the Ca^{2+} is increased in steps from 0.6 to 3.6 mM, an increase in e.p.p. amplitude with increase in Ca^{2+} is seen until at 3.6 mM Ca^{2+} no effect of the peptide on e.p.p. amplitude is seen: curare (1.5 mg l^{-1}) was added to the Ringer to prevent muscle twitching in response to nerve stimulation at high Ca^{2+} levels. In other experiments, in curare-free Ringer, no changes in m.e.p.p. frequency were seen when the peptide at 10^{-4} M was applied to the bath in Ringer containing 3.6 mM Ca^{2+} . The effect of increased calcium concentrations on the second phase of the response was not tested in these experiments. In addition to the above neurophysiological findings of a calcium dependency, a substance P-mediated reduction in ^{45}Ca uptake by isolated synaptosomes and an increase in ^{45}Ca sequestration by

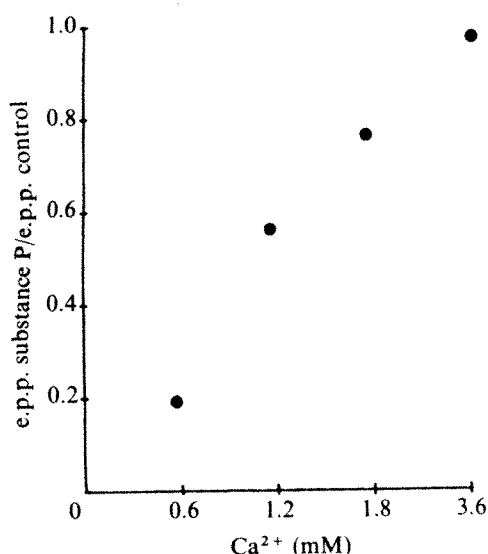


Fig. 3 Calcium dependence of the initial phase of synaptic depression produced by substance P. As calcium is increased, the reduction in e.p.p. amplitude produced by the peptide is decreased until, at twice normal Ringer calcium concentration (3.6 mM), the peptide has no effect on e.p.p. amplitude. Substance P concentration is 10^{-4} M. Each point is the average of five trials.

mitochondria isolated from rat brain has been found (unpublished data, A. Blitz, K. Dasse, and D. Sack). Effects on calcium uptake at these two sites both act to reduce free calcium in the presynaptic terminal. This, together with the above demonstrated blocking of the initial phase peptide effects by increased calcium in the Ringer, suggests that the synaptic depression seen in response to substance P is calcium dependent.

A possible mechanism of action for the substance P action reported here is a modulation of calcium-dependent potassium conductance which has been shown to regulate firing rates in several systems¹⁰⁻¹³. When delayed rectification is blocked in the frog neuromuscular junction, regenerative calcium dependent responses are seen in the presynaptic region¹⁴. A calcium-mediated potassium conductance has been suggested as a regulator of rhythmic and long term neural processes¹⁵. That substance P may be a physiological means of regulating this process is consistent with the above data. One could then postulate that the peptide functions to bias the output produced by the more static short term information transfer of the conventional transmitters.

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Postsynaptic receptors are not essential for dopaminergic feedback regulation

ANTIPSYCHOTIC drugs (neuroleptics) which block central dopamine (DA) receptors increase the synthesis and metabolism of striatal DA and the firing of dopaminergic neurones in the substantia nigra. These effects have been postulated to be secondary to the drug-induced blockade of striatal postsynaptic DA-receptors and mediated by a negative feedback loop impinging on nigral DA-neurones^{1,2}. However, the existence of alternative mechanisms has been recently postulated³⁻⁵. In particular, it has been suggested that neuroleptics stimulate dopaminergic firing by blocking nigral DA-receptors, thus relieving DA-neurones from the inhibitory action of DA released from dopaminergic dendrites (self-inhibition)⁶. Indirect support for this possibility derives from the recent discovery of a DA-sensitive adenylate cyclase within the substantia nigra⁷⁻⁹. We report here that destruction of striatal DA-sensitive adenylate cyclase by kainic acid¹⁰ fails to reduce the effect of neuroleptics and of apomorphine, a DA-receptor agonist¹¹, on striatal DA-metabolism. These data indicate that an interaction with postsynaptic DA receptors is not essential for the changes in DA-metabolism produced by neuroleptics and apomorphine and provide *in vivo* evidence for the existence of a dopaminergic negative feedback independent from postsynaptic DA-receptors.

Male Sprague-Dawley rats (290-310 g) were anaesthetised with Equithesin and placed on a stereotaxic frame (Kopf); kainic acid (Sigma) dissolved in 1 μ l of saline was injected in 2 min in the caudate of one side while that of the other side received the same volume of saline. Coordinates were A 2.2, V 2.8, L 5.0 of Pellegrino and Cushman¹².

Ten days post-surgery, rats were killed and the striata of each side dissected out and homogenised in Tris-maleate buffer for adenylate cyclase assay¹³ or frozen at -20°C until analysed for DA and 3,4-dihydroxyphenyl acetic acid (DOPAC)¹⁴ in order to estimate DA-metabolism¹⁵. The brains of three rats were perfused with formaldehyde, cut by cryostat into 40- μ m slices and stained by cresylviolet in order to ascertain the extent and topography of the lesion.

Haloperidol (Janssen) and fluphenazine (hydrochloride salt, Squibb), were injected intraperitoneally (i.p.) while apomorphine, hydrochloride, Sandoz) was administered subcutaneously (s.c). Protein was measured by the method of Lowry *et al.*¹⁶ using bovine serum albumin as a standard.

Figure 1 is a micrograph of rat striatum from the side injected with kainic acid (3 μ g 10 d previously), as compared with the contralateral side injected with saline. It seems that in the side injected with kainic acid neuronal perikarya are markedly reduced in number while only a slight gliosis is present. The loss of neuronal perikarya affected more than 80% of the caudate-putamen, the nucleus interstitialis striae terminalis and about 60% of the nucleus accumbens, but did not involve the adjacent cerebral cortex and thalamus.

As soon as the rats awakened from anaesthesia they showed continuous turning contralateral to the kainic-injected side and intermittent whole body-rocking. This symptomatology lasted for at least 3-4 h. By 24 h post-surgery, rats displayed homolateral torsion of the body and turning which, although diminished, was still present 10 d post-surgery. At this time, administration of apomorphine (0.1 or 0.5 mg per kg body weight s.c.) stimulated the homolateral turning while haloperidol (0.3 mg per kg i.p.) reversed the turning into a contralateral one.

In agreement with the results of McGeer *et al.*¹⁰ we found that the unilateral intrastratial injection of 3 μ g of kainic acid resulted in a 40% decrease of basal adenylate cyclase activity in homogenates of kainic-injected striatum. Moreover, in these homogenates DA completely failed to stimulate the adenylate cyclase activity even when added at a concentration of 100 μ M, which maximally stimulated adenylate cyclase of striatal homogenates from the control side.

Unilateral administration of kainic acid did not modify DA-concentrations but resulted in a 100% increase of DOPAC levels in the lesioned striatum (see Fig. 2).

Parenteral administration of haloperidol increased, and of apomorphine decreased DOPAC levels both in the kainic-injected and in the control striatum. As Fig. 2 shows, 90 min after a maximally effective dose of haloperidol (0.3 mg per kg), DOPAC concentrations increased by the same amount in the kainic-injected and in the control striatum, thus reaching significantly higher levels in the lesioned striatum. A lower dose of haloperidol (0.100 mg per kg) caused a much greater accumulation of DOPAC in the kainic injected striatum than in the control side and the absolute increase of DOPAC in the kainic-injected side was more than twice that in the control one. Finally, a dose of haloperidol of 0.030 mg per kg failed to produce a significant increase of DOPAC in the control

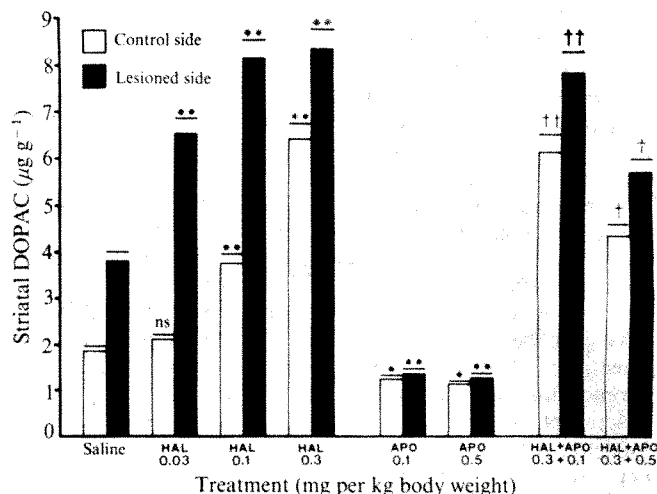
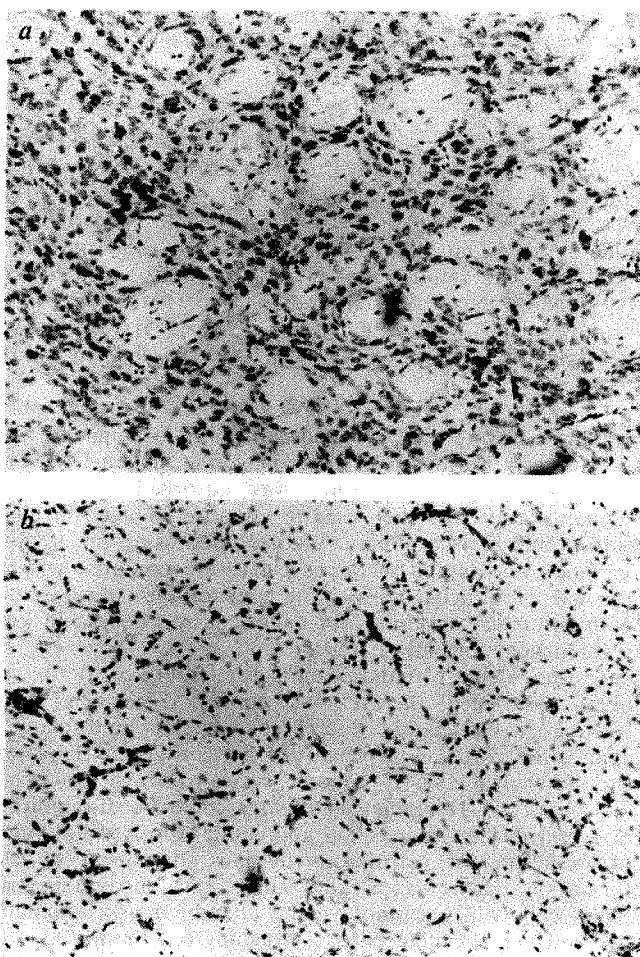


Fig. 2 Effect of haloperidol (H) and apomorphine (A) on DOPAC concentrations in control and kainic-lesioned striatum. Rats were injected with 3 μ g of kainic acid in the right striatum and with saline in the left one. After 10 d, rats were given haloperidol or apomorphine and were sacrificed 90 and 45 min respectively after drug administration. Each value is the mean $\pm$ s.e.m. of the number of determinations indicated in parentheses. * P < 0.01; ** P < 0.001, ns (not significantly different), in respect to the values of the homolateral striatum of rats administered with saline. † P < 0.01, ††, not significantly different, in respect to the values of the homolateral striatum of rats administered with haloperidol alone.

Fig. 1 Photomicrographs ($\times 5.25$) of a cresylviolet-stained section from a rat injected unilaterally in the striatum with kainic acid (3 μ g) 10 d earlier. *a*, Control striatum; *b*, kainic-injected striatum.



striatum but increased by about 100% the levels of DOPAC in the lesioned striatum. Similar results were obtained after 0.5, 0.2 and 0.05 mg per kg of fluphenazine.

Apomorphine administration (0.5 and 0.1 mg per kg; s.c.) produced a more pronounced decrease of DOPAC in the kainic-injected than in the control side. Finally, as shown in Fig. 2, apomorphine (0.1 mg per kg), failed to decrease DOPAC levels both in the kainic-injected and in the control striatum of rats pretreated with haloperidol (0.3 mg per kg). On the other hand, a higher dose of apomorphine (0.5 mg per kg) partly reversed the increase in DOPAC produced by haloperidol (0.3 mg per kg) in the lesioned and in the control striatum.

The complete loss of striatal DA-sensitive adenylate cyclase does not prevent the effect of DA-receptor blockers, as haloperidol and fluphenazine, and of a DA-receptor agonist, as apomorphine, on DA-metabolism. Since haloperidol and apomorphine were mutually antagonistic, their effects on DOPAC in the kainic-injected striatum should be mediated by an action on DA-receptors. Taking the loss of striatal DA-sensitive adenylate cyclase as demonstrating destruction of postsynaptic DA-receptors¹⁰, our results indicate that an action at the level of the postsynaptic DA-receptors is not a pre-requisite for the *in vivo* effects of DA-receptor blockers and agonists on DA-metabolism and strongly suggest the existence of an alternative dopaminergic mechanism exerting an inhibitory influence on DA-neurons.

The exact location of this mechanism remains to be established. It might be pre-junctional, being mediated by DA released from DA-terminals on to presynaptic DA-receptors regulating tyrosine hydroxylase^{15,16} and DA-release¹⁷ or located within the substantia nigra and mediated by DA released from dopaminergic dendrites on to DA-receptors localised on dopaminergic neurones⁶ or on connections afferent to the substantia nigra²¹.

A further interpretation of our data could be that the changes produced by the drugs in the kainic-injected striatum are mediated by an action on postsynaptic DA-receptors of non-striatal dopaminergic areas left intact by

the kainic treatment. This possibility is made unlikely, however, by the fact that the lesion produced by the intra-striatal injection of 3 μg of kainic acid was not restricted to the caudate-putamen but also involved other dopaminergic areas as the nucleus accumbens, the bed nucleus of the stria terminalis and the central nucleus of the amygdala. Prefrontal cortex was not found to be damaged but the paucity¹⁸ and uncertainty¹⁹ of the cortical afferences to the substantia nigra makes it difficult to admit that this area mediates the changes observed on the kainic-injected striatum. The tuberculum olfactorium was not affected by kainic acid but nigral afferences from this area have not been described¹⁸. Finally, the finding that the sensitivity of striatal DA-metabolism to neuroleptics and apomorphine is increased in spite of the large loss of brain DA-receptors produced by kainic acid is difficult to be interpreted as due to the activity of a residual extra-striatal negative feedback loop.

Note added in proof: A paper from Garcia-Munoz *et al.*²² has recently appeared, in which a different approach was used to reach the same conclusions of the present one.

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Hydroxyproline-containing protein adsorbed on to cellular elements of whole human blood

CURRENT hypotheses suggest that blood clotting occurs when, as a primary event, platelets come into direct contact with collagenous fibrils which have been exposed in vessel walls. We report here that a collagen component is normally associated with the cellular elements of blood. It was demonstrated by analysing whole blood and its fractions for protein-bound hydroxyproline, and shown by immunofluorescence in clotted blood smears using a specific antiserum to human collagen. As a blood component it is distinct from and additional to Clq, the hydroxyproline-containing protein which is a part of the first component of complement.

Hydroxyproline was first measured in serum and plasma by LeRoy¹ and was believed to be related to a soluble collagen component of blood. It was later found that this hydroxyproline was more probably related to Clq, which shares amino acid sequences including hydroxyproline, with collagen^{2,3}. The total serum concentration of hydroxyproline attributable to Clq, assuming a concentration of Clq in serum of 180 $\mu\text{g ml}^{-1}$ (ref. 4) and a hydroxyproline content of 5.4%, is 9.7 $\mu\text{g ml}^{-1}$ (ref. 5). Recent studies of whole citrated blood have shown about double this amount of hydroxyproline to be present, that is, 18 $\mu\text{g ml}^{-1}$ (ref. 6). The discrepancy between the serum and whole blood concentrations of hydroxyproline is apparently entirely due to collagen, which is all bound to blood cells.

Blood was drawn from three women (ages 25-52 yr) and three men (ages 23-67 yr) and hydroxyproline measured by the method of Woessner⁷ in whole blood, plasma and serum (Table 1). In agreement with the literature, plasma (or serum) has less than 10 $\mu\text{g ml}^{-1}$ bound hydroxyproline, so that the additional 10 $\mu\text{g ml}^{-1}$ originates from proteins

Table 1 Hydroxyproline levels in whole human blood and blood fractions

	Whole citrated blood ($\mu\text{g ml}^{-1}$)	Serum ($\mu\text{g ml}^{-1}$)	Plasma ($\mu\text{g ml}^{-1}$)	Plasma + cell washings* ($\mu\text{g ml}^{-1}$)	Cells† ($\mu\text{g ml}^{-1}$)
Means for six subjects	18.0	8.0	8.1	18.1	25.0
Range	15.4-23.3	6.8-10.5	7.7-11	15-20	21.2-38.8

*Additional and separate group of five subjects.

†Cell mean value calculated on the basis of haematocrit; cell range includes additional values obtained by direct analyses of centrifugal cells.

bound or adsorbed on to cellular elements. This was also seen in direct analyses of centrifuged cells, hydrolysed *in toto*, and in determinations of plasma plus cell washings. (For washing, 1 ml of 0.9% NaCl was added to 1 ml of whole blood; this is mixed and centrifuged and the supernatant removed. The cellular precipitate was resuspended in 1 ml 0.9% NaCl, recentrifuged, and the supernatant was again removed. This was repeated three times. Hydroxyproline determinations were performed on the pooled supernatants.)

To ascertain whether this adsorbed hydroxyproline-containing protein represented a form of collagen or Clq, prepared antiserum to human collagen (collagen D, Fig. 1) was used^{8,9}. The antiserum was adsorbed with whole human plasma and then used to visualise collagen in blood smears and clots by immunofluorescence using the indirect method. Smears and sectioned clots were prepared without the use of anticoagulants. The preparations were treated with anticollagen antiserum followed by fluorescein conjugated goat anti-rabbit serum⁸.

In the clots, a prominent diffuse reticulum of fluorescent fibrils interlaced the cellular masses (Fig. 2c,e). The smears showed highly fluorescent platelets and platelets aggregated (Fig. 2a). The clotted extremities of the smear showed a brilliant, if delicate, version of the sectioned clot (Fig. 2b). Here platelet clumps could be distinguished at every fibrillar intersection and could represent the points of initiation for a collagen fibrillar component of clot formation. There was a faint fluorescence surrounding red blood cells which cannot yet be unambiguously attributed to collagen. Collagenase (Worthington) removed the fluorescence reaction from fibrils and platelet clumps (Fig. 2d). Sections were subjected to additional controls including blocking, pretreatment with control rabbit sera, and pre-adsorption of the anti-human collagen serum with the

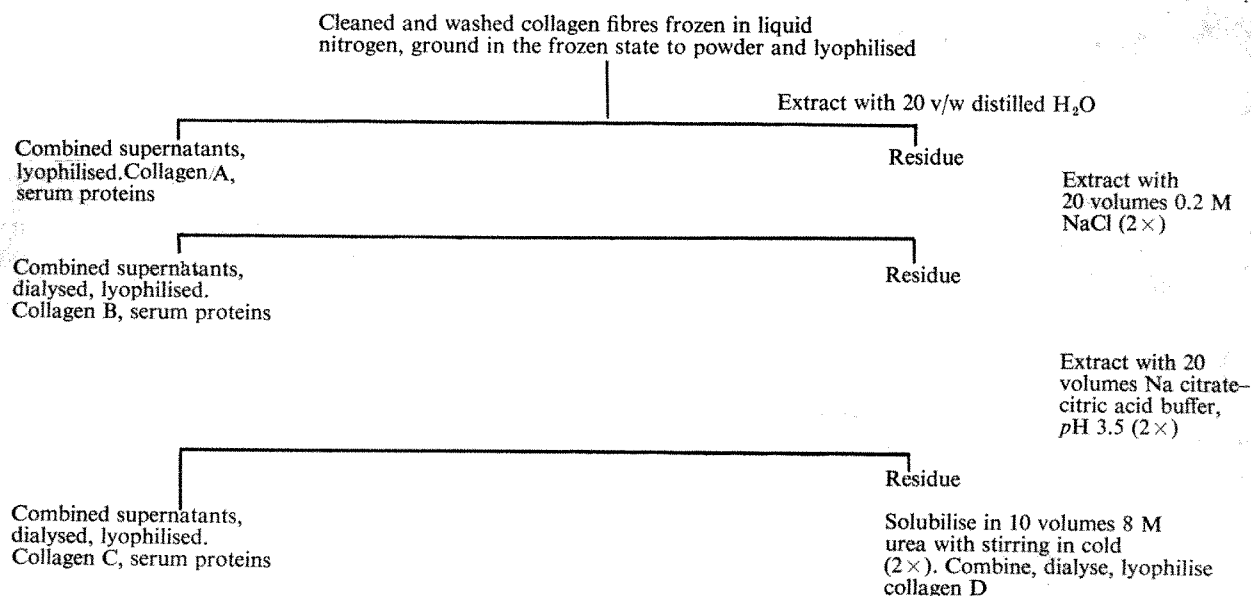
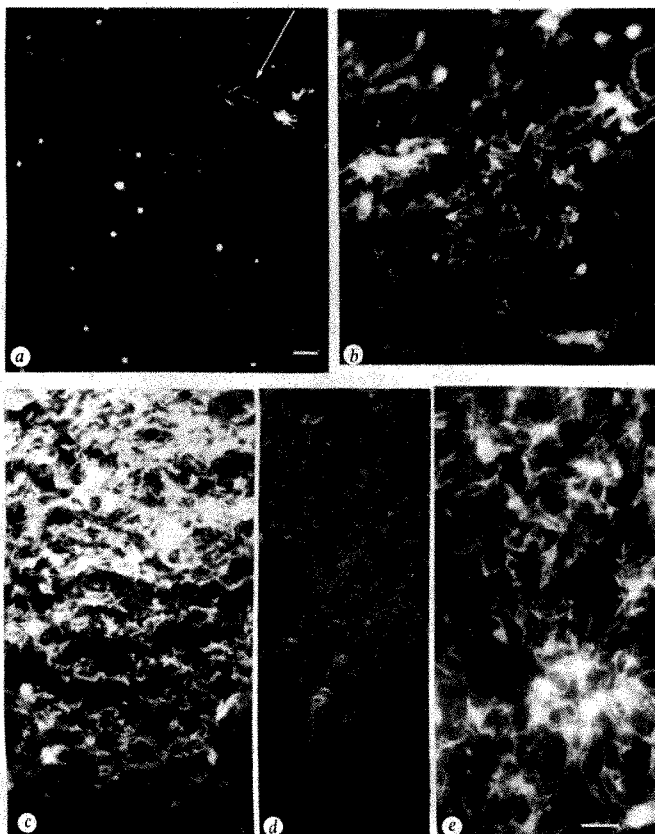


Fig. 1 Fractionation of human achilles tendon.

collagen antigen. Following these steps, the fluorescence reaction for collagen was negative.

The human collagen antiserum was extensively evaluated for specificity. After adsorption with plasma it did not react with serum proteins nor with C1q (M.B.E., unpublished). Amino acid analysis of collagen D was similar, although not identical, to that for human skin collagen¹⁰.

Fig. 2 Immunofluorescence smear and clot preparations of normal human blood stained by the indirect method using a rabbit anti-human collagen serum and a goat anti-rabbit serum conjugated with fluorescein isocyanate. *a*, Blood smear showing platelet fluorescence. At arrow, fine threads of an incipient clot. *b*, Clotted portion of blood smear showing additional collagen component possibly radiating from platelet foci. *c* and *e*, Collagen staining of sectioned clots with maximal appearance of collagen reactive material. *d*, Collagenase control of sectioned clot. Scale bars, 10 μ m.



We had attempted to remove, as far as possible, serum protein contaminants from this preparation. There was evidence for the presence of an unusual post-histidine residue corresponding to the histidinohydroxymerodesmosine described in other insoluble forms of collagen¹¹. It is not known whether the antiserum distinguishes between fibrillar and monomeric collagen.

Isolation procedures to characterise the hydroxyproline-containing protein of blood have been begun by one of us (R.M. and Bezkorovainy, A.). Plasma and supernatants of washed cells were applied to a Sepharose 6B column, using H₂O as eluting medium. Two hydroxyproline-containing peaks were resolved, one appearing in the void volume (molecular weight 10⁶), the other at a molecular weight of 400,000. The eluting medium was changed to 0.1 M NaHCO₃ and two hydroxyproline peaks again appeared, at molecular weights of 400,00 and 300,000. We assume that the 400,000 peak is C1q, while the large protein is some form of collagen which dissociated when the buffer was changed.

The interaction of collagen with platelets *in vitro* has been extensively studied¹²⁻¹⁵, always, however, with the assumption that the *in vivo* source of collagen was subendothelial tissue exposed by vascular injury. The presence of a collagen-like protein already present on the cellular surface must modify this interpretation. Addition of aggregating agents to platelets in platelet-rich plasma showed an almost immediate involvement of amorphous surface material in interplatelet bonds¹⁶. These bonds strongly resemble those seen in Fig. 2*b*. Not only platelets, but leukocytes and erythrocytes interact and aggregate with fibrillar collagen *in vitro*¹⁷. The binding sites involved in this collagen interaction—at least in the platelet and possibly in the leukocyte—also bind C1q and in its presence collagen-induced aggregation is lost. There thus seems to be competition of collagen and C1q for the same binding sites¹⁸⁻²².

In early stages of clotting, a substantial collagen reaction is present on platelets, but a portion remains occult, appearing as insoluble highly reactive fibrils only in areas where clotting had begun and showing maximally in the sectioned clot. Thus an intriguing question is whether this part of the circulating collagen is normally associated with the erythrocyte surface and becomes reactive during clotting. We have not examined smears from anticoagulated blood by immunofluorescence.

The hydroxyproline-containing protein described here

resembles collagen in both antigenic and chemical qualities and is a physiological component of the formed elements of blood. As such, it is certainly involved in their interactions including clot initiation and the possible displacement of Clq from antigen-antibody complexes. This role, and its modifications in situations where collagen becomes abnormally mobilised from tissues, remains to be evaluated in the areas of blood sedimentation, clotting, haemostasis and thrombosis.

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Effect of a single amino acid substitution on stability of conformation of a protein

THE role of individual amino acid residues in stabilising protein molecules is of interest, especially in connection with the mechanism of thermoresistance of thermophilic bacteria¹ and in connection with the physicochemical basis of temperature sensitive mutants used widely in molecular biology studies. This problem has been approached mainly with the use of synthetic polypeptide², chemical modification of natural proteins³, or homologous proteins⁴⁻⁹ from different species. Many mutants of microorganisms are available from genetic studies, however, in which the mutant proteins differ from wild type only at a single amino acid position. Such proteins often differ markedly in stability. We describe here a study of the α -subunit of tryptophan synthetase of *Escherichia coli*. The data suggest that the stability of a protein may be highly correlated with the hydrophobicity of the amino acid residues at some suitable positions. Further studies of this type may clarify the role of individual amino acid residues in stabilising proteins.

Many mutants of *E. coli* producing an α -subunit of tryptophan synthetase with a single amino acid substitution have been isolated¹⁰, and α -subunits of tryptophan synthetase from some *E. coli* mutants have altered stability¹¹. Table 1 shows a comparison of heat inactivation of tryptophan synthetase α -subunits from mutant and wild-type strains. Marked effects were seen when Glu

at position 49 was replaced by Gln (strain *trpA11*) or by Met (*trpA33*); the α -subunit became more labile or more stable, respectively. These results confirm those reported by Maling and Yanofsky¹¹.

α -Subunits were purified from the wild-type strain and the mutant *trpA33*, respectively, using the method of Creighton and Yanofsky¹² with a slight modification. Purified samples gave a single band on polyacrylamide gel electrophoresis. Circular dichroism (CD) spectra (200-250 nm) of the purified α -subunits from the wild type and *trpA33* were mutually not distinguishable and coincided with the results of Heyn and Weisheit¹³ on the wild-type protein. The heat inactivation of the purified samples coincided with those of crude extracts. We can therefore assume that the difference in thermostability of crude enzymes in Table 1 reflects the difference in that of the α -subunit molecule itself, which

Table 1 Heat inactivation of α -subunit of tryptophan synthetase of *E. coli*

<i>trp</i> Strain	Position of amino acid substitution ¹⁰	Remaining activity (%)
A218	22 Phe → Leu	10 ± 1
A11	49 Glu → Gln	22 ± 3
A33	49 Glu → Met	88 ± 13
A446	175 Tyr → Cys	60 ± 1
A487	177 Leu → Arg	16 ± 7
A223	183 Thr → Ile	35 ± 2
A23	211 Gly → Arg	13 ± 2
A46	211 Gly → Glu	76 ± 13
A187	213 Gly → Val	12 ± 4
A78	234 Gly → Cys	40 ± 8
A58	234 Gly → Asp	85 ± 10
A169	235 Ser → Leu	30 ± 4
B8	(Wild type)	49 ± 4

Cells suspended in pH 8.0, 0.1 M Tris-HCl buffer were disrupted in a Branson Sonifier B-12 sonic oscillator. The supernatant solutions obtained by centrifugation for 2.5 h at 80,000g were used for the heat inactivation study. These mutant proteins are inactive in the conversion of indole-3-glycerolphosphate to tryptophan, but retain the activity for the conversion of indole to tryptophan in the presence of pyridoxal phosphate, serine, and β -subunit of tryptophan synthetase of *E. coli*¹⁴. This latter activity was measured by the disappearance of indole after reaction at 37 °C for 20 min¹⁴. The β -subunit was extracted from mutant strain *trpA2* of *E. coli*. Heat treatment was done for 20 min at 58 °C.

is due to replacement of a single amino acid residue. The conformational changes of the α -subunits on heating were examined by measuring the CD values at 222 nm, where the CD spectra of the α -subunits showed a large negative Cotton effect. After incubation for 60 min at 60 °C in pH 8.0, 0.1 M Tris-HCl buffer, the per cent of remaining ellipticity at 222 nm of α -subunits from the wild type and *trpA33* was 66.5 and 82.0% respectively. After cooling for 10 min at 25 °C, the ellipticity of α -subunits from the wild type and *trpA33* recovered to 81.5 and 94.0%, respectively. When 0.2 mM dithioerythritol was present in the sample solutions, changes of ellipticity on denaturation were similar to those seen in its absence, but the recovery of the ellipticity was stimulated remarkably in both wild-type and mutant proteins. These results show that the conformation of the α -subunit from *trpA33* is more stable to heat than that of the wild type. It must be pointed out that the activity of α -subunit may recover to some extent during assay (20 min at 37 °C) after heat treatment. Data on heat inactivation of enzymatic activity may therefore only partially reflect conformational stability of the α -subunit.

We examined urea denaturation of the two proteins as an indication of stability, and also as the simplest procedure¹⁵ for estimating the free energy change of unfolding. Figure 1 shows a comparison of urea denaturation of the purified α -subunits of the wild type and the mutant *trpA33* measured by the change in CD at 222 nm. The mutant *trpA33* protein was more resistant to urea than that of the wild type, corresponding to the relative stabilities on heat denaturation. From far-ultraviolet CD spectra, the two proteins could be considered to be completely denatured in 8 M urea. The denatured proteins were renatured by diluting (Fig. 1),

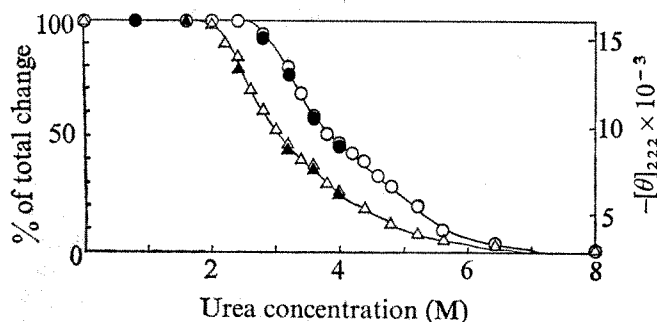


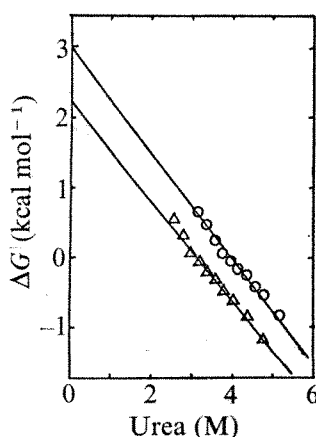
Fig. 1 Urea denaturation of the α -subunits of the wild type (Δ , $\blacktriangle$) and the mutant *trpA33* ($\circ$, $\bullet$). Temperature 25 °C, pH 8.0, 0.025 M Tris-HCl buffer. The solution contained 0.1 mM EDTA and 0.2 mM dithioerythritol. Open symbols represent the ellipticity at 222 nm after incubation for 60 min in urea solutions at various concentrations. Filled symbols represent the measurement of the samples diluted after exposure to 8 M urea solution to demonstrate the reversibility of the denaturation. CD measurements were carried out with a Jasco J-20 recording spectropolarimeter.

and enzymatic activity recovered completely. Urea denaturation is thus reversible in these conditions. The midpoints of urea denaturation curves at 25 °C were 3.1 and 3.9 M urea for the wild type and the mutant *trpA33*, respectively.

We analysed the data shown in Fig. 1 assuming that the urea denaturation can be approximately represented by an equilibrium between a unique native form and a completely unfolded form^{8,9,15}. Figure 2 shows the dependence of the free energy change of unfolding on the urea concentration. By extrapolating to zero concentration, the free energy changes of unfolding in water for the α -subunit of the wild type and the mutant *trpA33* are calculated to be 2,290 and 3,040 cal mol⁻¹, respectively. The difference between the wild type and the mutant *trpA33*, 750 cal mol⁻¹, amounts to about 30% of the free energy change of unfolding for the wild type.

X-ray diffraction studies have shown that the replacement of Glu 49 by Met caused little alteration of the conformation of the α -subunit¹⁶. Our results, taken together with the above data, show that a single amino acid substitution increased the stability of the molecule without a gross change in conformation. The side chain contribution of an amino acid to free energy change for transfer from ethanol to water has been estimated to be 1,300 and 550 cal mol⁻¹ for Met and Glu, respectively¹⁷. The difference between them, 750 cal mol⁻¹, coincides with the difference of the free energy change of unfolding of the wild type and the mutant *trpA33* proteins obtained here. This suggests that the increase in stability

Fig. 2 Effect of urea concentration on the free energy change of unfolding for α -subunits of the wild type (Δ) and the mutant *trpA33* ($\circ$). ΔG for two-state transition is given by the following equation; $\Delta G = -RT \ln K$. An equilibrium constant, K = (concentration of unfolded molecule)/(concentration of native one), is calculated from the data shown in Fig. 1, using only those points where K is between 0.2 and 5.0 (ref. 15). The lines in the figures were calculated by the method of least squares.



of the mutant *trpA33* protein compared with the wild type might be accounted for by the difference of hydrophobicity of Glu and Met, if we assume that the position 49 is somehow buried in the hydrophobic interior of the molecule. Although hydrophilic residues such as glutamic acid are usually located on the surface of protein molecules, there are exceptions, especially when the residues are in the active sites of enzymes¹⁸. In the case of Glu 49 of the α -subunit of tryptophan synthetase, its substitution by Met results in loss of activity with indole glycerolphosphate and serine as substrate, though not with indole and serine¹⁴. We may therefore assume that it is in the active site of the enzyme and hence in a largely hydrophobic interior environment, which may be simulated by ethanol or similar solvents, and in which partners for hydrogen bonding are at hand.

Our results suggest that the stability of an enzyme may be greatly increased by the increase in hydrophobicity by substitution of a few suitable amino acid residues. Stability of enzymes from thermophiles could be explained by similar mechanism.

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Preponderance of synonymous changes as evidence for the neutral theory of molecular evolution

ACCORDING to the neutral mutation-random drift hypothesis of molecular evolution and polymorphism^{1,2}, most mutant substitutions detected through comparative studies of homologous proteins (and the nucleotide sequences) are the results of random fixation of selectively neutral or nearly neutral mutations. This is in sharp contrast to the orthodox neo-Darwinian view that practically all mutant substitutions occurring within species in the course of evolution are caused by positive Darwinian selection³⁻⁵. This paper shows that by comparative studies of messenger RNA (mRNA) sequences reliable estimates can be obtained of the evolutionary rates (in terms of mutant substitutions) at the third positions of the codon, and that the estimates conform remarkably well with the framework of the neutral theory.

Salser *et al.*⁶ have presented a comparison of homologous parts from the fragments of the human and rabbit haemoglobin mRNA sequences. Among 53 nucleotide positions that can be compared, there are six base differences, of which only one leads to amino acid difference. Their Table 5 shows that among 17 third nucleotide positions of the codon which can be compared

between the two species, there are 5 nucleotide differences. To estimate the number of nucleotide substitutions per site (K) that have occurred in the course of evolution since the divergence of the rabbit and human lineages, I use the following formula which converts the observed difference into the estimate of evolutionary divergence

$$K = -\frac{3}{4} \log_e(1 - 4/3 \lambda) \quad (1)$$

In this formula, λ is the observed fraction of the sites by which two homologous sequences differ from each other (for details, see ref. 7); K includes superimposed and reverted mutant substitutions. The rate of nucleotide substitution per site per year can then be obtained by $k_{\text{nuc}} = K/(2T)$, where T is the time (yr) since the divergence of the two lineages. Also, the standard error (σ_K) of K may be computed using a formula given in ref. 7. Letting $\lambda = 5/17$ in equation (1), we obtain $K = 0.373$. The corresponding standard error (σ_K) is 0.182. Thus, taking $T = 8 \times 10^7$, we obtain $k_{\text{nuc}} = (2.3 \pm 1.1) \times 10^{-9}$ (the present estimate should be statistically more reliable than the corresponding estimate given in ref. 6). This is a very high evolutionary rate, comparable with that of the fibrinopeptides. If we denote by k_{aa} the rate of amino acid substitutions per site, it is known that for fibrinopeptides $k_{\text{aa}} = (4 \sim 9) \times 10^{-9}$ (see refs 8, 9). This figure, when converted into the nucleotide substitution rate, is $k_{\text{nuc}} = (1.8 \sim 4.0) \times 10^{-9}$. In addition, a direct comparison of human and rabbit fibrinopeptide A (using data given in ref. 8) gives $k_{\text{aa}} = 5.2 \times 10^{-9}$ leading to $k_{\text{nuc}} = 2.3 \times 10^{-9}$.

A similar but more interesting report comes from Grunstein *et al.*¹⁰ who compared histone H4 (that is, histone IV) mRNA sequences of two sea urchin species *S. purpuratus* and *L. pictus*. It is well known that histone H4 is by far the most highly conserved protein (with $k_{\text{aa}} = 0.006 \times 10^{-9}$ (ref. 8)). Their report¹⁰ now shows, however, that many synonymous changes (that is, the changes that do not lead to amino acid changes) have occurred in the gene coding for this protein in the course of evolution. From their Table 3, showing 84 nucleotide sites that can be compared between the messenger sequences of the two species, there are ten base differences, of which nine are located at the third positions of the codon. Actually, there are 27 third nucleotide positions which can be compared between these two species and there are nine nucleotide differences. Thus, we obtain $K = 0.440$ and $\sigma_K = 0.163$. According to Grunstein *et al.*, these sea urchins shared a common ancestor approximately 6×10^7 yr ago. Therefore, letting $T = 6 \times 10^7$, we obtain $k_{\text{nuc}} = (3.7 \pm 1.4) \times 10^{-9}$ as an estimate of the average evolutionary rate at the third nucleotide position of the codon in the histone H4 gene. It is remarkable that, in this gene, synonymous mutant substitutions have occurred with the highest known rate, in spite of the fact that the amino acid substitutions have occurred in the corresponding protein with the lowest known rate.

How do these observations bear relation to the neutral theory? Let k be the rate by which mutant genes are substituted in the species in evolution. If a certain fraction f_0 of the molecular mutants are selectively neutral and the rest are definitely deleterious (assuming that definitely advantageous mutations are negligible in frequency), then for neutral alleles, the rate of mutant substitutions is equal to the mutation rate¹. Therefore

$$k = \nu_T f_0 \quad (2)$$

where ν_T is the total mutation rate. Now, among the prominent features¹¹ of molecular evolution the following two are particularly noteworthy: (1) for a given protein, the rate of evolution is roughly constant per year and (2) the molecules or parts of molecules that are subject to less functional constraints evolve (in terms of mutant substitutions) faster.

As to the first feature, the neutralists assume that for a given molecule f_0 is roughly constant. Recently, some authors, such as Fitch and Langley¹², have emphasised the non-constancy, but

their results show, in agreement with our earlier estimate¹³, that the variance of evolutionary rates among lines is only about 2.6 times as large as that expected from chance fluctuations. It is possible that there is delicate fluctuation of intrinsic evolutionary rate around the mean, as caused by shifting of the molecular constraint as amino acids are substituted one after another in various parts of the molecule. This means that the probability f_0 of a mutation being neutral is not strictly constant but fluctuates around its characteristic mean. Also, as pointed out by Ohta¹⁴, if nearly neutral but very slightly deleterious mutations are prevalent, the evolutionary rate fluctuates as the population goes through a series of bottlenecks. As to the difference of evolutionary rates among molecules or parts of molecules, the neutralists assume that the probability of a mutational change being neutral depends on functional constraints. Namely, the weaker the functional constraint, the larger the probability (f_0) of a random change being selectively neutral, with the result that k in equation (2) gets larger. According to this explanation, the maximum evolutionary rate is attained when $f_0 = 1$, that is, when all the mutations are neutral. Now, the high evolutionary rates observed at the third position of the codon can be explained from the neutral theory by assuming that the majority of synonymous changes are selectively neutral. Note that roughly 2/3 of random nucleotide substitutions at the third position of the codon are synonymous. The possibility of synonymous nucleotide changes being selectively neutral was discussed extensively in an early paper² on the neutral theory. Of course, it is possible that not all synonymous mutations are completely neutral, but the possibility is very high that, on average, synonymous changes are subject to natural selection very much less than the mis-sense mutations. On the other hand, if we adhere to the selectionist position that practically all the mutant substitutions in evolution are caused by positive natural selection, there can be no upper limit to the evolutionary rate at the molecular level (as directly set by the mutation rate ν_T), and there is no reason to believe that the rate of evolution is uniform even approximately for a given molecule among different species.

In my opinion, various observations suggest that as the functional constraint diminishes the rate of evolution converges to that of the synonymous substitutions. If this is valid, such a convergence (or plateauing) of molecular evolutionary rates will turn out to be strong supporting evidence for the neutral theory.

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Inheritance of recombinant HLA-GLO haplotype suggesting the gene sequence

THE HLA region in man has been assigned to the short arm of chromosome 6 (ref. 1). Close genetic linkage has been shown between *HLA* and genes involved in the synthesis of the second^{2–3} and fourth⁴ components of complement as well as structural variation of the glycine-rich B-glycoprotein (GBG or Bf)⁵. Two enzymes, phosphoglucomutase-3 (PGM₃)^{6–7} and glyoxalase I (GLO)^{1,8–12}, and two blood group antigens, Chido¹³ and Rodgers¹, have been shown to be in this linkage group. Here we describe a

Table 1 M. family MLC testing stimulating cells

Responding cells HLA genotypes				A ×	B ×	C ×	D ×	E ×	F ×	Unrelated pool†	Reference stimulation‡
A	Father (TM)	A29-B12 c.p.m.*		8,225	99,415	95,191	91,244	96,729	90,928	90,046	81,818
		RR		0	87	106	101	108	101	100	
		A2-B12 SIR		1	9.7	11.6	11.1	11.8	11.1	10.9	
B	Mother (MM)	A2-B21 c.p.m.		51,554	3,792	49,346	61,240	48,821	48,739	83,394	79,603
		RR		60	0	57	72	57	57	100	
		A32-B7 SIR		13.6	1	13.0	16.0	12.9	12.9	22.0	
C	Sibling I	A29-B12 c.p.m.		61,135	20,893	992	64,033	66,456	44,478	73,454	72,462
		RR		83	28	0	87	90	60	100	
		A32-B21 SIR		61.6	21.1	1	64.5	67.0	44.8	74.0	
D	Sibling II (CM)	A2-B12 c.p.m.		42,000	17,952	37,808	2,508	7,499	10,934	65,151	48,526
		RR		63	25	56	0	0	13	100	
		A32-B7 SIR		16.3	7.0	14.7	1	2.9	4.2	25.3	
E	Sibling III (MM)	A2-B12 c.p.m.		45,654	11,956	16,886	7,784	1,991	1,485	51,518	63,007
		RR		91	19	49	10	0	0	100	
		A2-B21 SIR		15.3	4.0	9.0	2.6	1	0.5	17.2	
F	Sibling IV (MaM)	A2-B12 c.p.m.		57,514	19,058	38,047	9,053	4,999	2,068	65,075	31.5
		RR		88	27	57	11	5	0	100	
		A2-B21 SIR		27.8	9.2	18.4	4.4	2.4	1	31.5	

RR, relative response = $\frac{\text{test values (c.p.m.)—autologous stimulation (c.p.m.)}}{\text{response to unrelated pool—auto. stim (c.p.m.)}}$

SIR, stimulation index ratio = $\frac{\text{test value (c.p.m.)}}{\text{autologous stimulation (c.p.m.)}}$

*Tritiated thymidine incorporation.

†Pool of three unrelated controls.

‡Pool—autologous control.

family with an intra-HLA recombinant sibling and an HLA-GLO recombinant sibling. The mother is heterozygous at the HLA-A and -B loci, homozygous for HLA-DW2, and heterozygous at the GLO locus, thus permitting identification of an HLA-GLO recombinant in one of her children. Our investigations suggest that the locus for GLO polymorphism is on the centromeric side of HLA-B on the short arm of chromosome 6.

Table 1 illustrates the family mixed lymphocyte culture results¹⁴ showing that the lymphocytes of the mother only weakly stimulated those of her children, but in the reciprocal MLC reactions, her lymphocytes responded briskly to their. The sero-identical siblings III and IV are non-responsive in mutual MLC reactions, indicating identity at HLA-D. Interestingly, sibling II shows low mutual MLC responses with the HLA-D identical siblings III and IV.

These observations were corroborated by the HLA-D typing (Table 2). The family members were tested against homozygous typing cells representing the HLA-DW1-4 clusters as defined by the Sixth International Histocompatibility Workshop 1975; two HTC (FS and KL) which do not fall within the presently defined DW clusters, and one HTC (JB-12) which corresponds to the specificity

LD 107. The HLA-D typing results were analysed by the relative response and cluster methods⁴. The mother types as a HLA-DW2 and seems to be homozygous. The siblings II, III and IV are HLA-D identical (DW1-DW2) and sibling I inherited an HLA-A to -B recombinant (HLA-DW2-LD-107).

Slightly higher MLC reactivity than one would expect with complete HLA-D identity is observed when sibling II serves as either stimulator or responder to the sero-identical siblings III and IV. Although all three siblings type as HLA-DW1, DW2, the maternal haplotypes apparently carry slightly dissimilar DW2 determinants. This interpretation is consistent with evidence previously presented that the HLA-D region is not governed by a single gene, but more probably by a group of closely linked genes. Thus, the respective maternal D determinants may be overlapping or one may represent an inclusion of the other determinant⁴. Alternatively, the two maternal haplotypes may carry the same major HLA-D determinants, but differ at minor determinants at another locus¹⁵ or may differ at response genes¹⁶. Furthermore, back stimulation may be present to minor HLA-D determinants¹⁷.

The Bf typing¹⁸ gives rise to three common phenotypes

Table 2 Chromosome 6—gene markers of M family

Subject	Relationship	Haplotype designation	HLA-A*	HLA-B*	Bf	HLA-D	GLO	CHIDO
TM	Father	a	A2	B12	S	DW1	2	Ch (a+)
		b	AW29	B12	F	JB-12	2	
MM	Mother	c	AW32	B7	S	DW2	2	Ch (a+)
		d	A2	BW21	S	DW2	1	
BM	SibI	b	AW29	B12	F	JB-12	2	
		c-d (HLA-A-B recomb)	AW32	BW21	S	(LD-107) DW2	1	
CM	SibII	a	A2	B12	S	DW1	2	Ch (a+)
		c	AW32	B7	S	DW2	2	
MiM	SibIII	a	A2	B12	S	DW1	2	Ch (a+)
		d,c (HLA-D/GLO recomb)	A2	BW21	S	DW2	2	
MaM	SibIV	a	A2	B12	S	DW1	2	Ch (a+)
		b	A2	BW21	S	DW2	1	

*HLA determined by lymphocytotoxicity (see ref. 21).

(SS, FS and FF) determined by the relative mobility of the slow or fast migration bands to the anode of the electrophoretic plate. The father and sibling I demonstrates the FS phenotype; the mother and siblings II, III and IV the SS phenotypes. Thus, the F component is linked to the HLA-AW29, B12 haplotype of the father. Since the mother is homozygous for the S determinant, the Bf typing is not informative in following the crossovers involving the maternal chromosomes in the recombinant siblings I and II.

Glyoxalase-1 (EC 4.4.1.5) catalyses the formation of S-lactoyl glutathione from glutathione and methylglyoxal. Genetic polymorphism of this enzyme in red blood cell lysates was first reported by Kompf, *et al.*¹⁹. They described three different starch gel patterns representing homozygosity and heterozygosity for two alleles, GLO¹ and GLO², with frequencies of about 0.40 and 0.60, respectively. The method of Kompf, *et al.*¹⁹ was used for this study, except a phosphate buffer was used²⁰. Since the enzyme is a dimer, the heterozygote pattern has three major zones (two homodimers and one heterodimer), while the homozygote patterns contain only one major band each. Thus, the phenotypes are unequivocal, even though they are difficult to photograph, because the sites of catalytic activity appear as a lighter shade on the surrounding darker blue background. Figure 1 shows the three common phenotypes.

Several investigators^{1,8-10} have reported genetic linkage of GLO and HLA on chromosome 6. Due to the inability to establish linkage between PGM₃ and GLO, Kompf¹¹ suggested that GLO might be located on the side of the HLA-D locus at a distance of about 15 map units. The

sex-dependent difference in recombination frequency noted by Lamm *et al.* for PGM₃ was not observed by Weitkamp *et al.*⁹ for GLO, an observation consistent with the postulation by Kompf¹¹ that GLO may be located in the HLA-A side of the chromosome. Subsequent family studies, however, show GLO segregates with HLA-B^{1,9-10}. Weitkamp *et al.*¹ in studies of linkage between HLA, Bf and GLO suggested the GLO is closer to Bf than to HLA. Meera Khan *et al.*¹ in other linkage studies suggested that GLO is situated between PGM₃ and HLA. Furthermore, from the estimated recombination frequency between HLA and GLO of 0.15 (ref. 8), Pretorius *et al.*¹⁰ postulated the GLO locus to be located outside of HLA-D.

Assuming the least number of recombinants in the family described herein, the GLO locus seems to be outside the locus for HLA-B. The placement of the GLO locus on the centromeric side of HLA-B is strengthened by the observation of MLC non-reactivity between siblings III and IV indicating their HLA-D identity. Thus, the maternal haplotypes are apparently HLA-AW32-B7-DW2-GLO2 and HLA-A2-BW21-DW2-GLO1. Sibling III inherited a recombinant haplotype HLA-A2-BW21-DW2-GLO2 with the recombination occurring somewhere between the HLA-B and GLO. Our investigation therefore suggests that the most probable order of loci on the short arm of chromosome 6 proceeding toward the centromere is: HLA-A, HLA-B, HLA-D, GLO.

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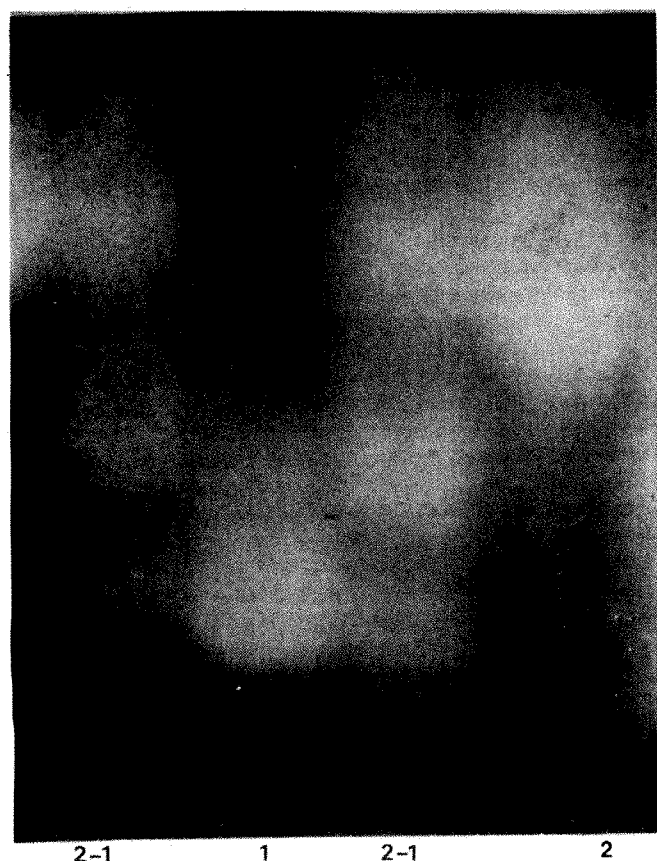
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Fig. 1 Starch gel electrophoretic patterns of the three common glyoxalase-1 phenotypes, GLO 1, 2-1, and 2. The sites of enzyme activity are lighter coloured than the darker staining background.



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Structure-function relationship of 5' non-coding sequence of rabbit α - and β -globin mRNA

RETICULOCYTES make essentially equimolar amounts of α - and β -globin as required for the correct assembly of haemoglobin. It has been shown, however, that α and β mRNAs differ in their rate constant for ribosome attachment^{1,2}. In particular α -globin mRNA initiates protein synthesis only 60% as frequently as β -globin mRNA (refs 3–5). The 5' non-coding region is thought to be involved in the binding of the mRNA to the ribosome for the correct initiation of protein synthesis. A comparative study of the sequences of the 5' non-coding regions of α - and β -globin mRNA may show whether their differential rate of initiation can be ascribed to specific features in the mRNA structure or whether it is a function of some other part of the translational machinery. Recently the complete sequence of the 5' non-coding region of rabbit β -globin mRNA has been elucidated⁶. I report here the complete sequence of the 5' non-coding region of rabbit α -globin mRNA, determined following essentially the same methodology.

A ³²P-labelled complementary DNA (cDNA) of the 5' non-coding region of both α - and β -globin mRNA was prepared using the synthetic deoxyhexanucleotide primer d(G-C-A-C-C-A) complementary to the codons for the Met-Val sequence common to both proteins and avian myeloblastosis virus (AMV) reverse transcriptase.

The cDNA was fractionated by one-dimensional homochromatography (see Fig. 1a). Bands 2, 3 and 5 were identified as cDNA of the 5' non-coding region of β -globin mRNA by their characteristic depurination fingerprints⁶. Bands 1 and 4 were tentatively classified as cDNA of the 5' non-coding region of α -globin mRNA because their intensity relative to the β bands increased when a template enriched in the α component

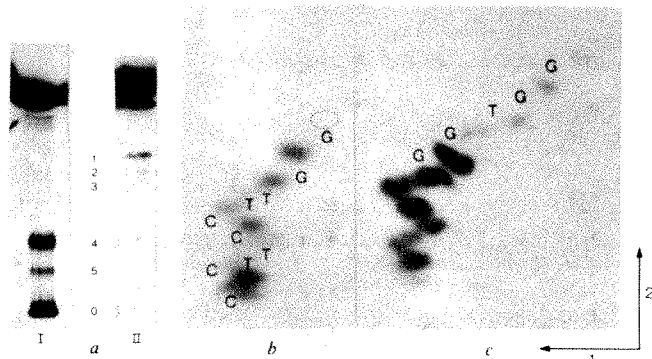


Fig. 1 *a*, Autoradiograph of a one-dimensional homochromatography fractionation of cDNA (ref. 7). Radioactive cDNA was prepared *in vitro* with AMV reverse transcriptase using each α -³²P-labelled deoxynucleoside triphosphate (dNTP) of high specific activity in turn and the other three unlabelled. The synthesis was carried out with all four dNTPs present (I), or in the absence of dATP (II). O denotes the origin, and the strong band at the top is unreacted dNTP. The reaction conditions were identical to those previously described⁶, except that the primer used was the hexanucleotide d(G-C-A-C-C-A) and the template was a mixture of α - and β -globin mRNA (a gift from Dr N. J. Proudfoot) obtained from the post-ribosomal supernatant that had been shown to be enriched in the α component⁸. AMV reverse transcriptase was a gift of Dr J. W. Beard. Bands 1 and 4 are cDNA of α -globin mRNA 5' non-coding region; band 1 ends before the first dA residue and band 4 is the full length copy. Bands 2 and 5 are cDNA of β -globin mRNA 5' non-coding region; band 2 ends before the first dA residue and band 5 is the full length copy. *b*, *c*, Two-dimensional fingerprints⁹ of a partial venom phosphodiesterase digest of band 1 labelled with dGTP (*b*) or dTTP (*c*). The sequence d(5') [T]G-G-T-G-G-T-T-C-C-T-T-C-T-C(3') was deduced from the relative mobilities of the intermediates. The absence of radioactive products after the last G shift in (*c*) is interpreted as the next residue being the dT adjacent to the primer.

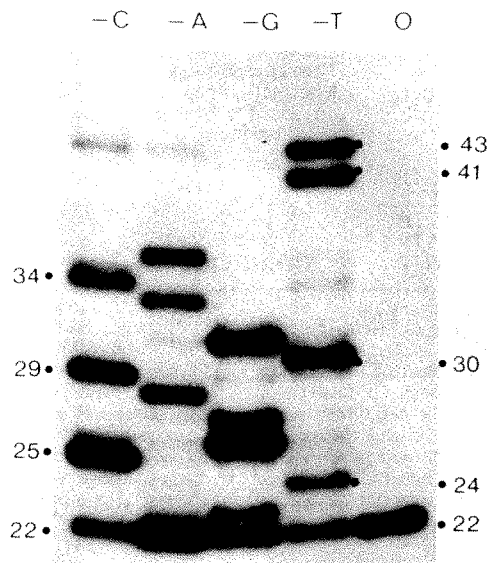
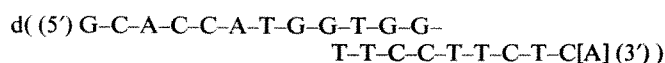


Fig. 2 Gel analysis of partially minus reactions. Band 1 was eluted from the homochromatography⁷, desalted through a Sephadex G-100 column and freeze dried. The cDNA was then dissolved and re-annealed with mRNA. This preparation was divided into four aliquots, and 50 nM 'minus' reactions⁶ were performed. Each 'minus' mixture contained three dNTPs at 50 μ M concentration and one dNTP in turn (the limited one, so called 'minus') at 50 nM concentration. In this way it was possible to obtain from a single product (band 1) specific 'pile-ups' of radioactivity in positions corresponding to the limiting dNTP. The reaction mixtures were fractionated in 7 M urea 12% polyacrylamide gel. The slots marked -C, -A, -G, -T denote that the reaction analysed was carried out with the corresponding dNTP at a concentration of 50 nM. Slot O is a control lacking the enzyme. The bands correspond to positions in the sequence where the limiting dNTP is present. The numbering refers to the cDNA sequence shown in Fig. 3. Longer exposed films clearly showed bands at position 38–39 in the -A slot and at position 32 and 42 in the -G slot.

was used. Definitive evidence for this assignment is discussed below.

Nearest neighbour analysis carried out for band 1 showed that only when the label was α -³²P-dTTP was radioactive dAp found; dCTP and dGTP failed to label dAp (see Table 1). As band 1 was synthesised in the absence of dATP, and therefore lacked any internal dA residues, it was concluded that the last residue of the primer was the one labelled, as expected if the initiation codon was A-U-G. Depurination¹⁰ of bands 1 and 4 provided the data summarised in Table 1. Partial digestion with venom phosphodiesterase of the cDNA of band 1 was carried out; the two-dimensional fingerprint is shown in Fig. 1 *b* and *c*. The cDNA sequence d((5') G-G-T-G-G-T-T-C-C-T-T-C-T-C(3')) can be read off from the characteristic mobility shifts⁹. Note the sharp difference in spot intensity after the first G shift. This unusual pattern may be an indication of some structural feature (hairpin loop?) in this short cDNA oligonucleotide.

Combining data from depurination, nearest neighbour analysis and partial venom phosphodiesterase digestion, the sequence of band 1 cDNA was established as



To obtain the sequences between the end of band 1 and the end of band 4 (possibly the full length cDNA) a new gel sequencing technique was used¹⁹. This is an adaptation for RNA sequencing of the 'plus' and 'minus' system developed for Φ X DNA sequencing¹¹. It consists of the extension of a mixture, as heterogeneous as possible, of different transcript lengths in the complete absence of each one of the four dNTPs in turn. In this case a slight variation was introduced to overcome the problem

Table 1 Depurination and nearest neighbour analysis data from bands 1 and 4 cDNA (Fig. 1a)

Band no.	Input label	Base composition of depurination products (deduced from their position)	Nearest neighbour analysis data			
			C	A	G	T
1	dGTP	T[G]	0	0	1	1
1	dTTP	T ₂ C ₄ *, T	2	1	2	2
1	dCTP	T ₂ C ₄ *	1	0	0	3
4	dATP	C ₂ [A]; T ₂ C ₄ [A]*	—	—	—	—
4	dGTP	T[G]; T C[G]	—	—	—	—

Depurination data—5' and 3' terminal phosphates of depurination products are omitted. [] denotes that the base within brackets was deduced by nearest neighbour considerations. Thus depurination products obtained from α -³²P-dGTP or α -³²P-dATP input label oligonucleotides contain G or A residues respectively on their 3' sides. Nearest neighbour analysis—this section is divided into four columns indicating the products of analysis Cp, Ap, Gp and Tp. These are denoted by C A G T, respectively. Ratios of product yields were established by scintillation counting of the spots.

*Base composition deduced from the partial venom digests shown in Fig. 1b.

of nonspecific priming. The extension of a single product (the α -globin mRNA specific product—band 1) was carried out in partially minus conditions (each of the four dNTPs, in turn, at limiting concentrations—see Fig. 2). The band pattern produced allowed the reading of the cDNA sequence as d((5') A-G-T-C-G-G-A-C-T-G-G-A-C-C-A-X-A-A-X-T-G-T (3')) where X represents unknown residues, their presence deduced by the spacing of the bands. The last ten residues overlap with the sequences already reported for the 5' end of α -globin mRNA¹³, thus confirming the identity of the priming site. It is a ten-base overlap with two ambiguities. Because of the clear overlap it was not necessary to establish the nature of the

X residues in this study as they had been defined by others. The complete cDNA sequence deduced is shown in Fig. 3a.

The 5' non-coding regions of α - and β -globin mRNA (Fig. 3b) are substantially dissimilar in size and nucleotide composition. These sequences have only a few common features; residues 1–6 are identical¹³ and there is a purine-rich region centred six to eight residues to the left of the A–U–G initiating codon.

It has been suggested⁶ (although critical evidence is still lacking) that the region around the A–U–G initiating codon of β -globin mRNA and brome mosaic virus (BMV) RNA interact with the 3' terminal sequence of 18S rRNA. This is similar but not identical to the interaction between the 5' non-coding region of mRNA and 16S rRNA in bacteria^{14,15}. For α -globin mRNA the same interaction is possible at positions 37–41 (Fig. 3), involving the first two triplets of the coding region (common to both messengers) but forming one base pair less than β -globin mRNA. Another possible region of interaction with 18S rRNA is at nucleotides 8–12 (Fig. 3), but this is less attractive for two reasons. First, in the known bacterial examples¹⁵ the interaction of the mRNA with 16S rRNA occurs at a maximum distance of 10 bases from the initiation codon, while here if an interaction were to occur it would be 24 bases away. Second, it has been shown that the A–U–G is in a rather central position of the ribosome-protected fragment in the case of β -globin mRNA^{16,17}. It should be noted that residues 8–12 are in the stem of the proposed hairpin loop (see below).

An obvious problem with the proposed mRNA–18S rRNA interaction around the initiation codon is that both the Met-tRNA_i and the 18S rRNA would compete for the A–U–G sequence of the mRNA. This would be unlike the situation in *Escherichia coli* where the interacting site of the mRNA is displaced approximately ten nucleotides to the left of the initiation codon. The difficulty may be resolved if the two interactions occur sequentially rather than concurrently. Other parts of the extensive 5' non-coding region may also be involved

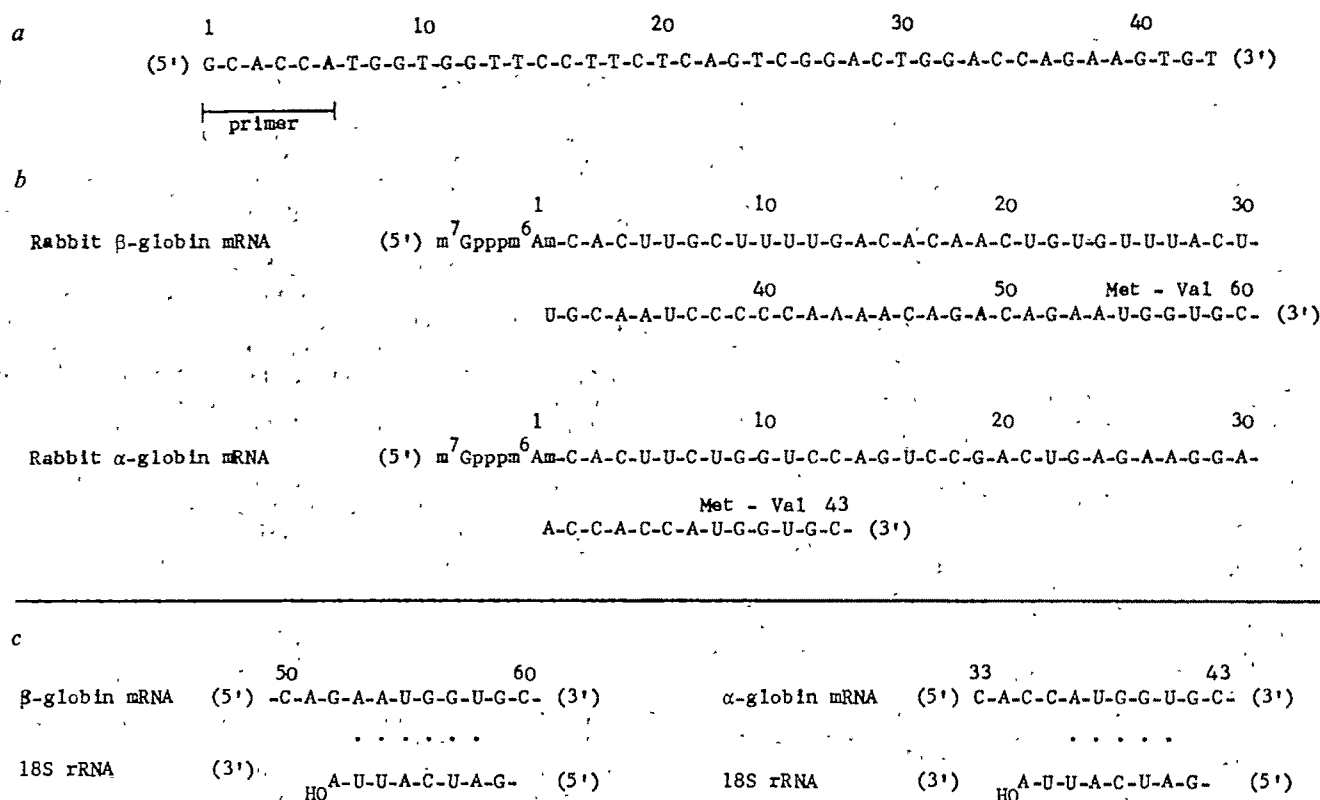


Fig. 3 a, Sequence of the cDNA. Numbers indicate the distance of the nucleotide at that position from the 5' end of the primer. The sequence between positions 34 and 43 has been previously reported¹³. b, Sequence of the 5' non-coding region of rabbit α - and β -globin mRNA. Numbers indicate the distance of the nucleotide at that position from the 5' m⁷Gppp structure. c, Base pairing of α - and β -globin mRNA with the known 3' end sequence¹³ of rabbit 18S rRNA.

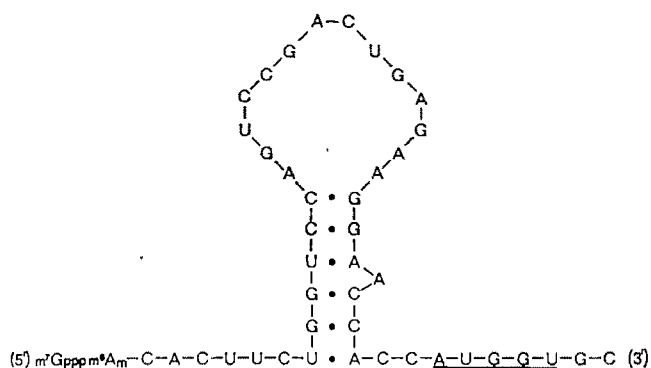


Fig. 4 Possible conformation of the 5' non-coding region of α -globin mRNA. The estimated stability of the hairpin loop is discussed in the text. The underlined sequence is complementary to the 3' end of rabbit 18S rRNA (see Fig. 3).

in other RNA-RNA interactions; for example, by base pairing with internal positions of 18S rRNA.

A number of possible secondary structure models can be drawn by maximising the extent of base pairing. In particular for α -globin mRNA a hairpin loop can be constructed (Fig. 4) that has a large negative free energy (ΔG (25 °C) = -7.6 kcal), estimated according to the rules proposed by Tinoco *et al.*¹⁸. Other secondary structures can be drawn that partially 'mask' the postulated 18S rRNA interacting region (underlined in Fig. 4), but are less stable than the proposed loop. For β -globin mRNA there is no indication of stable secondary structure⁶. The best hairpin loop that can be drawn (not shown) has a negative free energy six times smaller than the α -globin mRNA conformation shown in Fig. 4 and is considered to be unstable, or only marginally stable. It is interesting to note that the α -globin mRNA hairpin loop (Fig. 4) involves about two-thirds of the 5' non-coding region, and most of the adjacent sequence is common with that of β -globin mRNA (the first six nucleotides and the first two codons, see Fig. 3).

The purpose of this study was to elucidate the sequence of the 5' non-coding region of α -globin mRNA. It was hoped that a comparison of this sequence with its β counterpart would provide a clue to understanding the molecular basis of their differential rates of initiation. The 5' non-coding regions of α and β -globin mRNA are 36 and 53 nucleotides long, respectively. They show few common features, making it difficult to pinpoint specific sequence differences that may be correlated with the differential rates of initiation. One significant point to arise, however, was that α -globin mRNA seemed to have a strong hairpin loop whereas β -globin mRNA did not. Thus a knowledge of the 5' non-coding sequence has not established the cause of the differential rate of initiation but has suggested that it might be caused by the differences in secondary structure.

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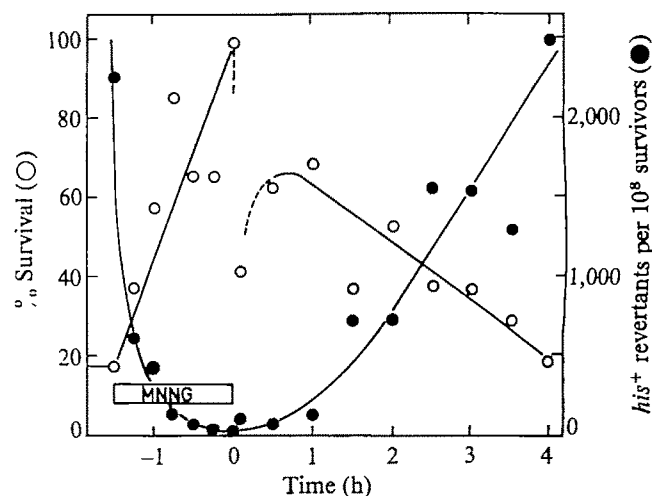
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A new pathway for DNA repair in *Escherichia coli*

MOST studies on mutagenesis involve exposing a growing population of cells to a large dose of mutagen for a short period. In a natural environment, however, cells are probably exposed more often to a low concentration of mutagens for long periods. We therefore measured the accumulation of mutants in *Escherichia coli* cells growing continuously in the presence of very low concentrations of mutagens. Because many mutagens are highly unstable, it was necessary to devise a system in which cells could be continuously exposed to fresh mutagen. To do this we took advantage of the fact that certain bacteria can grow and divide while attached to the underside of a Millipore filter. This system was originally developed by Helmstetter and Cummings¹ as a means of producing synchronous cells, because as growth medium is passed through the filter it carries away with it the unattached daughter from each pair of newly divided cells. The mutagen to be studied can therefore be stored in stable conditions and only mixed with the growth medium immediately before being passed through the filter; for example the mutagen *N*-methyl-*N'*-nitro-nitrosoguanidine (MNNG) can be stored in citrate buffer at pH 5.0 in which it has a half life of 40 h compared with 2.3 h in synthetic minimal media^{2,3}. We had intended to determine the relationship between ambient concentration of various mutagens and resulting mutation rate. The first mutagen studied was MNNG and the results were totally unexpected.

Fig. 1 The effect of previous growth in 1 $\mu\text{g ml}^{-1}$ MNNG on mutagenesis and killing by a short exposure to 100 $\mu\text{g ml}^{-1}$ MNNG. *E. coli* F26 (B/r., *thy his*) was grown at 37 °C with aeration to a density of 1×10^8 cells per ml in M63 medium⁴ supplemented with glucose (0.1% w/v), thymine (4 $\mu\text{g ml}^{-1}$) and histidine (40 $\mu\text{g ml}^{-1}$). At -1.5 h, MNNG (1 $\mu\text{g ml}^{-1}$) was added and samples were removed every 15 min till time zero; during this period the viable count increased 2.3-fold. At time zero, the culture was filtered and resuspended in the absence of MNNG, after which samples were removed every 30 min for a further 4 h. MNNG (100 $\mu\text{g ml}^{-1}$) was added to each sample for 5 min and the samples were then filtered, diluted and plated on nutrient broth plates and on M9 (ref. 7) plates containing thymine but not histidine. Survival (○) and *his*⁺ reversion (●) were measured. (The transient drop in survival of cells at time zero, indicated by interrupted lines, is apparently due to a transient increased susceptibility of cells to MNNG immediately after filtration.)



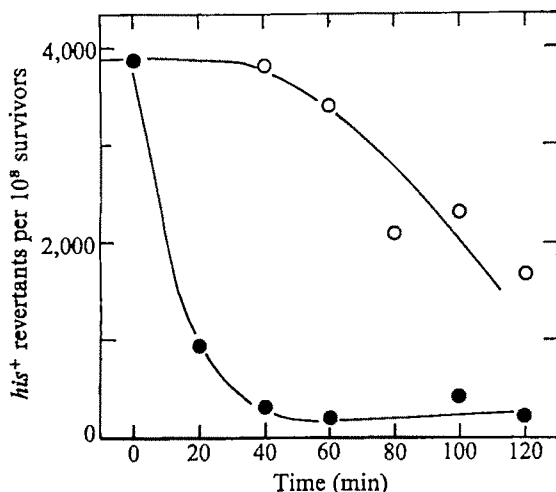
Bacteria on a filter could be kept growing for at least a week in a continuously replenished supply of medium containing $1 \mu\text{g ml}^{-1}$ fresh MNNG, and this produced a significant increase in frequency of reversion of a *his* nonsense mutation. The mutations arose only during the first hour of exposure to MNNG, however, and no further increase in frequency could be detected during the remaining 4–7 d of the experiment. (The technical details of these long term experiments will be published elsewhere and are not relevant here.) A similar result had been reported by Jiménez-Sánchez and Cerdá-Olmedo³ but they were more interested in where the mutations arose in the chromosome than in when they appeared during MNNG treatment, and so did not pursue the matter further. The simplest interpretation for our failure to collect an increasing number of mutants with time would be that the constant exposure to MNNG allows each bacterium to handle MNNG more efficiently and so become resistant to MNNG mutagenesis. Here we show that bacteria can indeed quickly become resistant to MNNG, apparently through the induction of a novel form of DNA repair.

The first and simplest experiment was to add $1 \mu\text{g ml}^{-1}$ MNNG to an exponentially growing liquid culture of *E. coli*. At various times thereafter samples from the culture were subjected to $100 \mu\text{g ml}^{-1}$ MNNG for 5 min and the resulting number of mutants and extent of killing were measured. The results clearly indicate that the bacteria had become increasingly difficult both to kill and to mutate during their growth in $1 \mu\text{g ml}^{-1}$ MNNG. On removal of the MNNG from the growing culture these properties decayed over a period of 4 h (Fig. 1). This effect looks very much like the induction and decay of a gene product and indeed, as Fig. 2 shows, the attainment of MNNG resistance did not occur in the presence of chloramphenicol and hence requires *de novo* protein synthesis.

Although we have not carried out any very extensive study of other mutagens, preliminary experiments have shown that a low level of MNNG induces resistance not only to MNNG itself but also to *N*-methyl-*N*-nitrosourea and ethyl methanesulphonate; it does not, however, induce resistance to ultraviolet light or methyl methanesulphonate.

The chloramphenicol-sensitive step that brings resistance to MNNG could be the synthesis of a protein which operates in one of two ways. The protein could prevent lesions being formed in the cells, for example, by making

Fig. 2 The effect of chloramphenicol on the induction of MNNG resistance. At time zero MNNG ($1 \mu\text{g ml}^{-1}$) was added to two cultures of *E. coli* F26. Samples were removed every 20 min and challenged with $100 \mu\text{g ml}^{-1}$ MNNG for 5 min, and the number of induced mutations was measured. One culture received chloramphenicol ($100 \mu\text{g ml}^{-1}$) for the first 20 min (○) and the other did not (●). A control experiment had previously shown that such exposure to chloramphenicol had, on its own, no effect on the response to challenge with MNNG.



the cell impermeable to MNNG or by breaking down MNNG as it enters the cell; or it could allow cells to handle the lesions more efficiently, for example, by carrying out some form of DNA repair like the inducible SOS repair system described by Witkin⁴ and George *et al.*⁵

All the available evidence seems to suggest that the effect is due to the induction of a new pathway for DNA repair, and is not due to something that prevents lesions being formed. For example, preliminary experiments have shown that methylation of DNA by ³H-MNNG occurs at approximately the same rate in induced and non-induced cells (P. Jeggo, personal communication). In addition, the survival of transforming plasmid DNA (ref. 8) that has been alkylated by MNNG *in vitro* is greater if it is assayed in cells that have been induced by MNNG (Fig. 3). Thus the

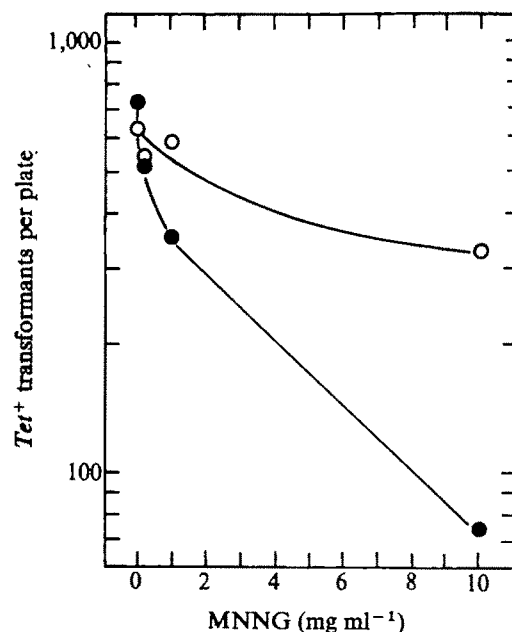


Fig. 3 The ability of transforming DNA, previously alkylated by MNNG *in vitro*, to transform induced and non-induced *E. coli* C803. MNNG was added to samples of tetracycline-resistant (Tet^r) PMB9 DNA (ref. 8) to 0.1, 1 and 10 mg ml⁻¹ at 37 °C for 25 min, and subsequently removed by dialysis overnight. These DNA samples were used to transform competent *E. coli* C803 cells (*gal*⁻, *met*⁻, *supII*⁻, *hsr*⁻, *hsm*⁻, *supIII*⁺) which were either induced (○) or non-induced (●) by 1 h of growth in $1 \mu\text{g ml}^{-1}$ MNNG. The efficiency of transformation was measured by the number of Tet^r transformants produced when competent cells were transformed by $1 \mu\text{g ml}^{-1}$ DNA.

function of the induced protein(s) is probably to repair damage caused by the mutagen. But, unlike the SOS repair system^{4,6} which allows increased survival at the cost of an increased mutation rate, this system lowers the mutation rate; further, preliminary experiments have shown that it involves a different set of gene products than SOS repair (P. Schendel, personal communication). We therefore believe that these results indicate the presence in *E. coli* of a DNA repair system, which is inducible but relatively error-free.

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Wide ranging plasmid bearing the *Pseudomonas aeruginosa* tryptophan synthase genes

THE wide ranging group of plasmids comprising the P compatibility class in *Escherichia coli* (ref. 1) (the P-1 class in *Pseudomonas aeruginosa* (ref. 2)) contains within it some members capable of serving as sex factors for the transmission of chromosomal markers from one *P. aeruginosa* strain to another. Haas and Holloway³ selected variants of one such P-1 plasmid, R68, that had become highly efficient in *Pseudomonas* chromosomal gene mobilisation. Some of these variants are also efficient sex factors in *E. coli* and *Rhizobium leguminosarum*⁴. Previous work with P-1 plasmids showed that they can incorporate resistance markers from *Pseudomonas*-specific plasmids and transmit them to *E. coli*⁵. We report here the analogous incorporation of the chromosomal *trpAB* genes from *P. aeruginosa* into R68.44, and a study of the expression of these genes after transfer to *E. coli* K12.

The tryptophan synthetic pathway and the genes responsible for its several steps in *E. coli* and *P. aeruginosa* are shown in Fig. 1. In *Pseudomonas* the two genes encoding the α and β chains of tryptophan synthase, *trpA* and *trpB*, are widely separated from the remaining pathway genes; this gene pair is regulated through induction by the substrate, indoleglycerol phosphate, rather than repression by L-tryptophan as is the case for certain other *Pseudomonas* *trp* genes and the entire *E. coli* *trp* operon⁷. Genetic studies^{8,9} have suggested that this regulation is autogenous and that the *trpA* gene product is at least part of the repressor, but a direct test of this hypothesis has been impossible because of the lack of a suitable source of DNA bearing the *Pseudomonas* *trpAB* region.

PAC174, a strain of *P. aeruginosa* requiring lysine and carrying the plasmid R68.44, was grown in nutrient broth

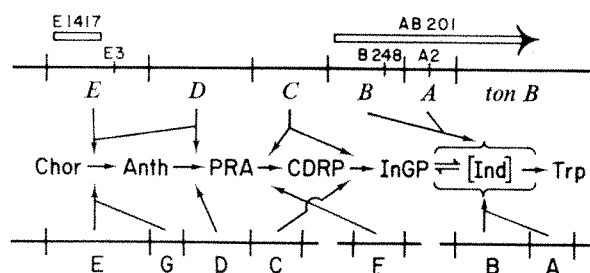


Fig. 1 Pathway of tryptophan (Trp) synthesis from chorismate (Chor). Intermediates: anthranilate (Anth), phosphoribosyl anthranilate (PRA), 1-o-carboxyphenylaminodeoxyribulose-5-phosphate (CDRP), indole-3-glycerol phosphate (InGP) and indole (Ind)[enzyme-bound]. Above the reactions the genes of the tryptophan operon of *E. coli* are drawn, arrows indicating the reactions their protein products participate in. Locations of the three *trp* mutations in strain IC762 are shown, along with the deletions ($\Delta trpE1417$ and $\Delta tonBtrpA201$) present in a double deletion strain in which a set of plasmid variants similar to those in Table 1 were also obtained. Because of poorer growth yields associated with the *tonB* character, enzymic analysis was performed only in the IC762 background. Below the reactions are shown the chromosomal *trp* gene distributions for *P. putida* and *P. aeruginosa*. (The location of the *trpG* gene is deduced from indirect evidence, since mutations affecting its gene product have not yet been reported.) Arrows indicate the reactions each gene produces catalyses.

together with *E. coli* W3110 resistant to rifampicin and carrying *trpA33*, a non-reverting mutation which produces an ineffective tryptophan synthase α chain¹⁰. After incubation at 37 °C for 3 d, the mixture was plated on minimal lactose agar with 50 $\mu\text{g ml}^{-1}$ of rifampicin. About one cell in 10^{10} formed a colony. All seemed indistinguishable and one clone, designated W3110*trpA33rif*^R (*ptrpAB*), was chosen for study.

It was a typical *E. coli* K12 with the antibiotic resistances and phage sensitivities characteristic of a strain carrying R68.44 (ref. 3). When R751 (ref. 11), a plasmid incompatible with R68.44, was introduced into W3110*trpA33rif*^R (*ptrpAB*), the drug resistances and the ability to grow without added tryptophan were lost. When *ptrpAB* was transferred to *E. coli* K12 strains carrying various *trp*⁻ mutations, it conferred prototrophy on those strains with defective *trpA* or *B* genes or with an extensive deletion involving these genes (Fig. 1) but not on those with mutations in *trpC*, *D*, or *E*.

Measurement of the molecular weight of covalently closed circular (CCC) plasmid DNA by sedimentation through a neutral sucrose gradient gave a value of 37.6×10^6 for R68.44 and 113×10^6 for *ptrpAB*. Thus, *ptrpAB* seems to carry at least 75×10^6 of DNA from the *P. aeruginosa* chromosome. Since the molecular weight of entire *P. aeruginosa* chromosome is about $2,100 \times 10^6$ (ref. 12), *ptrpAB* carries about 3.6% of it. *ptrpAB* is unstable; plasmid species conferring resistance to ampicillin, tetracycline and kanamycin and sensitivity to phages specific to P plasmids, but not containing the *trpAB* genes, can be transferred from *E. coli* (*ptrpAB*). Neutral sucrose gradient analysis of ³H-labelled *ptrpAB* CCC DNA (Fig. 2) shows,

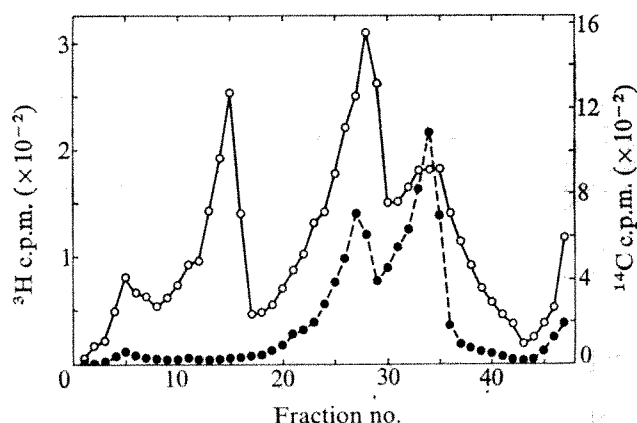


Fig. 2 Neutral sucrose gradient analysis of *ptrpAB* DNA. Covalently closed circular tertiary DNA forms of ³H-labelled *ptrpAB* and ¹⁴C-labelled R68.44 were isolated using caesium chloride-ethidium bromide density gradient centrifugation¹⁶. A mixture of the two plasmid DNAs was analysed by sedimentation through a 4.6-ml 5-20% sucrose gradient¹⁷ for 70 min at 100,000g and 20 °C. Fractions (0.1 ml) were collected directly on to glass fibre disks and, after drying, washing and redrying, were assayed for radioactivity. Sedimentation was from right to left. O, ³H; ●, ¹⁴C. The molecular weights of the plasmid species were calculated as described¹⁷.

in addition to CCC DNA of molecular weight 111×10^6 (fraction 15), a CCC DNA species of molecular weight similar to the CCC DNA of ¹⁴C-labelled R68.44 (fraction 28). The ¹⁴C-DNA peak at fraction 34 is open circular DNA of R68.44 formed by spontaneous single-strand scission of the CCC DNA. The ³H-DNA co-sedimenting with this peak probably represents the open circular form of the smaller DNA species found in *ptrpAB* DNA.

To study the expression of the plasmid-borne *trpA* and *trpB* genes we transferred *ptrpAB* into *E. coli* IC762, a derivative of W3110 that is *his*⁻, *str*^R, and bears three

mutations in the *trp* operon, *trpE3* (a mis-sense mutation inactivating the first tryptophan-specific enzyme), *trpB248* and *trpA2* (mis-sense and ochre mutation affecting the β and α subunits of tryptophan synthase, respectively). The intact *trpD* and *trpC* genes in this triple mutant allow anthranilate taken up from the medium to be converted to indoleglycerol phosphate, but no further along the pathway (Fig. 1). IC762 cells containing *ptrpAB* have the expected antibiotic resistance complement and require histidine and tryptophan. When provided with anthranilate in place of tryptophan they grow very slowly (>10 h generation time), although their copious excretion of ferric chloride-positive material indicates that availability of indoleglycerol phosphate cannot be limiting the growth rate. Extracts of cells grown in the presence of anthranilate have extremely low tryptophan synthase activity (Table 1). (For comparison, *P. aeruginosa* PAC21, the plasmid-free parent of PAC174 and hence donor of the *trp* genes on *ptrpAB*, grown in minimal medium has 0.70 tryptophan synthase units per mg protein.)

Table 1 Tryptophan synthase levels in cells harbouring R68.44 *ptrpAB* plasmids

Strain	Tryptophan	Culture conditions Anthranilate	Indole
IC762	< 0.02	No growth	No growth
IC762 (<i>ptrpAB</i>)	< 0.02	0.0	No growth
IC762 (<i>ptrpAB-1</i>)	< 0.02	0.70	No growth
IC762 (<i>ptrpAB-2</i>)	0.02	1.42	No growth
IC762 (<i>ptrpAB-3</i>)	2.57	2.09	2.07
IC762 (<i>ptrpAB-4</i>)	0.08	0.83*	0.72

Strain IC762 is an F⁻*his^rstr^r* derivative of the triple mutant *trpE3 trpB248 trpA2* constructed and characterised earlier¹⁴. Plasmid-bearing cells were grown 16–20 h at 37 °C with shaking in 1 l of minimal medium¹⁵ containing 0.2% glucose, 0.05% acid hydrolysed casein, and 4 mg of tryptophan or 10 mg of anthranilate or indole. Reversion controls showed the strains' growth properties to be unchanged at the time of collection. Cells were centrifuged, washed once in 0.1 M potassium phosphate, pH 7.5, suspended in 5 ml of the same buffer and disrupted by sonication. Extracts were assayed for tryptophan synthase (indole → tryptophan) and protein as described^{7,8}. Specific activity is given in units (0.1 μ mol per 20 min) per mg protein.

*This culture also contained 4 mg of tryptophan because the cells cannot grow on anthranilate alone.

We have not succeeded in isolating single-step variants of IC762 (*ptrpAB*) able to grow rapidly on anthranilate. After several serial transfers of 10-ml cultures in minimal medium containing anthranilate in place of tryptophan, however, we obtained a succession of faster growing strains whose improvements were plasmid-associated. Table 1 presents enzyme assays of two members of such a series, *ptrpAB-1* and *ptrpAB-2*. Growth rates conferred by these plasmids on several *E. coli* strains lacking tryptophan synthase are about proportional to the enzyme levels; that of IC762 (*ptrpAB-2*) growing on anthranilate is approximately 4 h. Enzyme levels are quite low in cells grown in the absence of anthranilate (Table 1) and the level of enzyme induced by anthranilate is not influenced by excess tryptophan (data not shown).

Strains lacking anthranilate synthase and having *ptrpAB* as their sole source of tryptophan synthase do not grow at all on low levels (5 μ g ml⁻¹) of indole in place of tryptophan. Even those containing *ptrpAB-2* do poorly (generation time >12 h). In this they resemble anthranilate synthase-less mutants of *P. putida*⁷ and *P. aeruginosa*¹³. We sought mutants of IC762 (*ptrpAB*) able to utilise indole at this concentration, for the *Pseudomonas* precedent^{7,8} suggests that such mutants may either be constitutive for tryptophan synthase or have mutations in *trpA* abolishing the ability to grow on anthranilate but permitting indole to act

as an inducer. Indole utilisers of both types were found (Table 1). In each case the regulatory change was shown to be plasmid-associated when the extrachromosomal element was transferred to other *E. coli* strains.

The tryptophan synthase produced by *ptrpAB-1*, *ptrpAB-2* and the regulatory mutants *ptrpAB-3* and *ptrpAB-4* was found to resemble that of *P. putida* and *P. aeruginosa* in its response to monovalent cations^{7,13} and was effectively neutralised by antiserum prepared to the β_2 subunit of the *P. putida* enzyme but poorly by antiserum to the *E. coli* β_2 subunit. One millilitre of antiserum against the β_2 subunit of *P. putida* tryptophan synthase neutralises 18 units of *ptrpAB-2* enzyme produced in IC762 or the double deletion background, and 36 units of PAC21 tryptophan synthase. Antiserum against *E. coli* β_2 subunit (homologous neutralisation 1,430 units ml⁻¹) gave only 4 units ml⁻¹ neutralisation against the plasmid-derived or the PAC21 enzyme. Clearly enzyme produced in *E. coli* by the plasmid genes is very similar to that of the *P. aeruginosa* parent, though more precise characterisation awaits its availability in greater quantity and purity.

We conclude that *ptrpAB* contains, in addition to the genes of R68.44, a rather large segment of the *P. aeruginosa* chromosome including the *trpAB* gene pair. The regulation of this gene pair in *E. coli* cytoplasm seems to be similar to that in *Pseudomonas* rather than the coliforms. Any gene products other than tryptophan synthase required for this regulation must be present on the 75 $\times 10^6$ segment of presumably contiguous chromosomal DNA carried by the plasmid. As first isolated, the plasmid's *trpAB* gene activity was low. Variants were found having more normal activity levels. The nature of these variants is not clear, but the existence of an impediment to the expression of certain *Pseudomonas* genes in *Escherichia* ought to be borne in mind when intergeneric recombinant DNA experiments are contemplated.

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Phonons and the elastic moduli of collagen and muscle

BIOLOGICAL fibres make a suitable subject for the development of the science of biomaterials. The amino-acid sequence of the collagen molecule is known^{1,2}, and has been used to account for the self-assembly of collagen into native fibrils¹ and polymorphic forms³, the pattern of bands due to positive staining in electron micrographs⁴ and the low-angle meridional X-ray diffraction pattern⁵. The collagen structure to amino acid resolution in axial projection is thus completely understood. Here we relate this structural picture to the mechanical properties (and since we are dealing with a biological fibre, these are also the biological properties) of the fibres. This can be done by investigating the vibrational and other motions of the molecular assembly in collagen. The force constants of hydrogen bonds in some polypeptides have been estimated on the basis of models⁶ but no experimental measure of their magnitude has been reported. We describe measurements of the inelastic light scattering (Brillouin) spectra of collagen from rat tail tendon from which we obtain elastic moduli of the tropocollagen molecule and an estimate of hydrogen bond force constants. We have also studied effects of hydration on the spectra to obtain information about the association of water with collagen in the native state. For comparison we have made preliminary measurements on molluscan 'catch' muscle in which the polypeptide conformation is different from collagen.

Samples of collagen were excised from tail tendons of adult rats and a bundle of fibrils ~0.2 mm in diameter mounted under tension sufficient to remove the characteristic banded appearance under the polarising microscope and produce alignment of the fibrils. The Brillouin spectra were recorded using a triple-pass Fabry-Perot interferometer (based on Burleigh Instruments model DAS-1/RC140) and were excited using between 5–50 mW of argon laser light at 514.5 or 488.0 nm. The incident and scattered beams were in the horizontal plane at 90° to one another and the sample orientated at 45° to these directions such that momentum transfer was along the fibre axis. In this configuration the wavevector transfer to the sample is independent of its refractive index and is equal to $2\sqrt{2}|k_0|$ where k is the wavevector of the laser light *in vacuo*.

Figure 1 shows spectra of a sample mounted in air at 12% relative humidity at 25 °C. There is a central feature due to elastically scattered light at the laser frequency and two inelastic components due to energy gain and energy loss. The

Fig. 1 Brillouin spectra for rat tail collagen fibres at 25 °C and 12% relative humidity showing the mode polarisation.

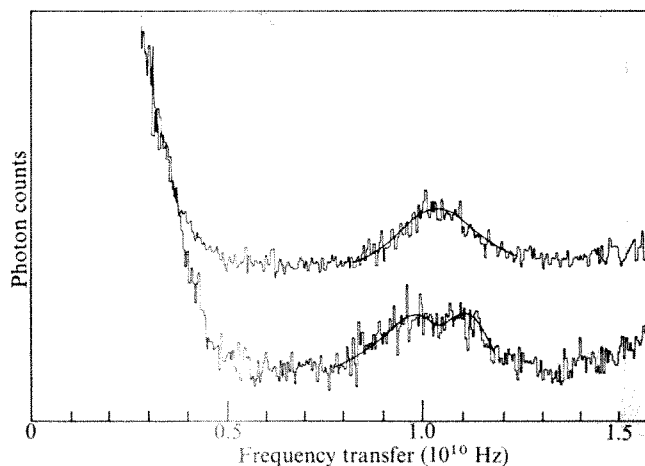
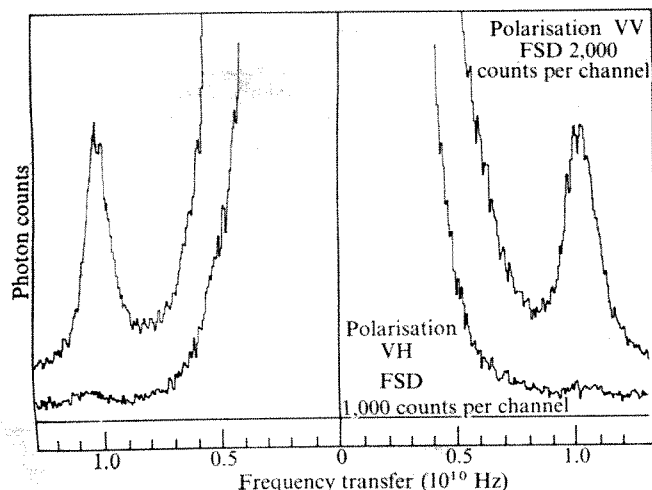


Fig. 2 Brillouin spectra from the air-dried anterior byssus retractor muscle of *Mytilus edulis* (at 25 °C) at two values of the relative orientation of the fibre and the scattered momentum transfer, q . Upper trace, q // fibre axis; lower trace, q 10° off axis.

frequency shift of these components varies linearly with laser frequency indicating that they correspond to acoustic phonons. The spectra in Fig. 1 are for two different polarisations of the incident and scattered light; VV refers to both polarisations vertical, that is, perpendicular to the scattering plane, and VH refers to incident polarisation vertical and scattered polarisation horizontal. The Brillouin components are of order 40-times stronger in VV than in VH polarisation; we can thus identify the scattering as due to longitudinal acoustic waves propagating parallel to the fibre axis⁷. No transverse acoustic modes have been observed at frequency shifts greater than 6.0 GHz despite an extensive search.

Figure 2 shows spectra from the anterior byssus retractor muscle of *Mytilus edulis*. The specimen had been dried in air at 25 °C and was mounted in the same manner as the dry collagen sample. Only one side of the elastic peak is shown; in the upper spectrum the sample is at exactly 45° to the incident laser beam and, by coincidence, Stokes and anti-Stokes Brillouin components from adjacent orders of interference overlap. In the lower spectrum the sample is tilted 20° towards k_0 such that wavevector transfer is about 10° from the fibre axis and the two Brillouin components are then just resolved because of the anisotropy in the acoustic velocity.

Figure 3 shows spectra for samples of collagen in 0.15 M NaCl solution; collagen in air at 100% relative humidity and for pure water in an equivalent scattering geometry. The measurement in 0.15 M NaCl was made with the fibre orientated as before but pressed between microscope slides, thereby ensuring that the fibre was bathed continually in the solution by capillary action. This arrangement prevents drying and gives the closest approach to the native condition of the collagen fibre (A. M. and B. B. Doyle, unpublished). For the measurement at 100% relative humidity the fibre was mounted in a cell at 25 °C through which humidified air was circulated for 2 d before the measurements started.

Figure 4 illustrates the result of experiments in which the collagen fibre was held in air at 25 °C at various relative humidities. The frequency of the longitudinal acoustic mode decreases as the humidity is increased and for high humidities approaches the frequency observed in 0.15 M NaCl (compare upper two spectra in Fig. 3). The intensity scales were different for each spectrum. In fact the Brillouin intensity is more or less unchanged over the whole range of humidities. The peak widths were instrumental. Spectra at different humidities were difficult to reproduce for two reasons. First, local heating by the laser beam dried the sample locally and thus the observed frequencies were power dependent; the spectra of Fig. 4 were obtained

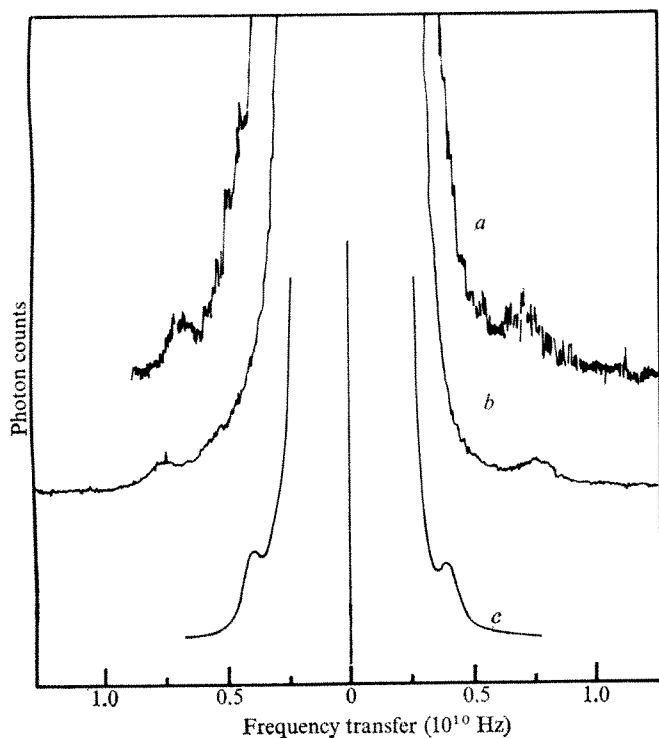


Fig. 3 Brillouin spectra from collagen *a* in 0.15 M NaCl solution between slides and *b* in 100% relative humidity vapour compared with *c*, the Brillouin spectrum of water. Brillouin scattering is from rat tail collagen laser heating effects.

using the lowest possible laser power (~ 5 mW) but even so at 84% and 100% humidity are appreciably affected by local drying, as can be seen by reference to Fig. 3. Second, hysteresis in the hydration of the fibres was observed; the frequency depended on whether a previously dried fibre was humidified or a wet fibre dried to a particular humidity. Experiments were performed to see if the Brillouin frequency of 84% humidity depended on external load attached to the fibre. The frequency was the same, within experimental error, for tensions between 1×10^7 and 4×10^8 dyn cm^{-2} .

Table 1 gives the measured longitudinal mode frequencies, corresponding acoustic velocities and elastic moduli, in various conditions. The calculation of elastic modulus requires knowledge of the density of the material. For dry collagen and muscle we estimate the density ρ from the excluded volumes of different amino acid residues present⁸ and their masses. For dry collagen $\rho = 1.43$ g cm^{-3} and for muscle $\rho = 1.40$ g cm^{-3} .

In the native state, assumed to be at 95% humidity, $\rho = 1.16$ g cm^{-3} (ref. 1) (assuming density of water = 1) for collagen. The amount of water present was taken from the adsorption isotherms for collagen¹⁰.

To discuss the sound velocities and moduli in collagen and muscle we take as a basis the calculations of Fanconi and Peticolas⁶ on poly-L-alanine. The paramyosin chain of the muscle, where the α helix is gently supercoiled with a second molecule, 3.5 residues per turn (1.485 Å repeat distance) as opposed to 3.6 in the α helix (1.5 Å repeat distance), is closely related to this synthetic α -helical polymer. The acoustic slope of the seven mass model poly-L-alanine can be fitted to the observed frequency and the calculated wavevector when one chooses a hydrogen bond force constant of about 0.07 m dyn $\text{\AA}^{-1}$. For synthetic macromolecules¹¹ the modulus decreases strongly with degree of coiling because bond twisting rather than bond stretching or angle opening strains accommodate the stress. This is presumably also the case for the polypeptide and so this hydrogen bonding force constant must, for the moment, be regarded as a fitting parameter. By the same principles, the higher modulus of collagen compared with muscle is consistent with the collagen conformation being more extended (3.3 residues per turn and 2.86 Å between residues). The moduli obtained by light scattering are about two orders of magnitude stiffer than the values derived from static measurements involving macroscopic extensions. This is again a familiar observation for synthetic polymers¹¹ and indicates inhomogeneous strain for measurements.

The sound velocity in collagen decreases monotonically with the degree of hydration (Table 1). Since the bulk density decreases on hydration it is supposed that both softening of the intrachain forces, particularly the hydrogen bonds, and rigid loading of the chains by the adsorbed water occurs. At 85% humidity there is about one water molecule adsorbed per peptide on the average¹⁰. At this point the mass per unit length of the chain has increased by 35% giving a frequency decrease of 17% compared with the 32% actually found. The decrease can be parameterised by first accounting for the decrease in sound velocity due to rigid loading, and then estimating the changes in H-bond force constant by interpolating the reduced sound velocities between values derived from the seven atom model calculations of Fanconi and Peticolas⁶ with a range of assumed H-bond force constants. This procedure gives the figures in the last column of Table 1. This approach is preferred to a discussion involving changes in the molecular conformation as a result of hydration since there is no detectable change in the high-angle X-ray diffraction pattern. At very high hydration the approach used here breaks down and the effects may be discussed using extended hydrodynamics¹².

In summary, measurements of the inelastic light scattering

Table 1 Acoustic properties of collagen and muscle at 25 °C

Substance	Q ($\text{\AA}^{-1}$)	v (GHz)	Sound velocity (cm s ⁻¹)	Density (g cm ⁻³)	Elastic modulus (dyn cm ⁻²)	H-bond force constant (m dyn $\text{\AA}^{-1}$)
Poly-L-alanine (theory)	0.18	1,800	6.4×10^8	1.51*	6.18×10^{11}	0.3
	0.23	1,800	4.8×10^8	1.51*	3.48×10^{11}	0.15
	0.23	258	6.9×10^4	1.51*	7.2×10^9	0.0
Muscle 12% relative humidity	1.727×10^{-3}	9.69	3.53×10^5	1.40*	1.74×10^{11}	0.1†
Collagen at 0% relative humidity	1.727×10^{-3}	10.65	3.88×10^5	1.43*	2.15×10^{11}	0.11‡
Collagen at 12% relative humidity	1.727×10^{-3}	10.29	3.74×10^5	1.4†	1.96×10^{11}	0.11‡
Collagen at 30% relative humidity	1.727×10^{-3}	9.00	3.27×10^5	1.38†	1.47×10^{11}	0.09‡
Collagen in 0.15 M NaCl solution (assumed equivalent to 85%)	1.727×10^{-3}	7.20	2.62×10^5	1.31†	9.0×10^{10}	0.08‡
Collagen (air dried) ¹³	(a) Macroscopic stress-strain measurements				$\sim 10^9$	
	(b) Using the 660-Å reflection to measure strain				$\sim 10^9$	
	(c) Using the 2.8-Å reflection to measure strain				$\sim 2 \times 10^9$	
Collagen (dry) ¹⁴	X-ray measurements				1.22×10^{10}	

*Assumed 0% humidity and density determined from peptide molecular weights and exclusion volumes¹⁵ from Chothia.

†Calculated from the adsorption isotherms. Rougvié and Bear¹⁰ by taking arithmetic mean density.

‡By interpolation between calculated values of sound velocity from Fanconi and Peticolas⁶.

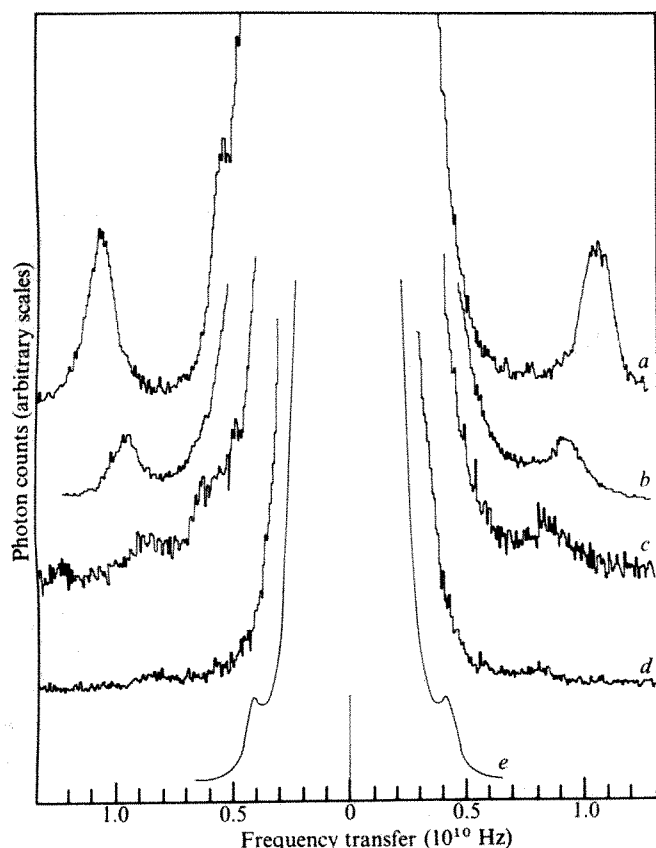


Fig. 4 Dependence of the Brillouin frequencies on degree of hydration for rat tail collagen at 25 °C. Obtained at lowest possible laser power at relative humidities of *a*, 0%; *b*, 30%; *c*, 84%; *d*, 100%. *e*, Spectrum of ordinary distilled water (not dustfree).

from collagen and muscle give values for the velocities of acoustic waves and elastic moduli in the medium. For dry collagen this leads to an estimate of the hydrogen bond strength. For material in the native state the measurements are capable of giving information about the dynamics of water molecules in the material.

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Crystal structure of anhydrous cholesterol

CHOLESTEROL, the most abundant steroid in the animal kingdom, is found mainly as a component in cell membranes and lipoproteins. The presence of the hydrophilic C(3) hydroxyl group in this otherwise hydrophobic molecule allows cholesterol to occupy a position at polar-nonpolar interfaces. Crystals of 3-hydroxy steroids and their hydrates show a tendency towards double layer structures with an end-for-end arrangement of approximately parallel molecules¹. As this molecular organisation is generally similar to that in membranes, it can be expected that some of the principles of intermolecular association in membranes might be revealed in the molecular packing in the crystal structures. Another common feature in these structures is the presence of more than one molecule in the crystallographic asymmetric unit. While this is a complicating factor in structure analysis, it clearly increases the amount of information regarding preferred conformation and intermolecular association to be gained from a structure determination. Craven² has recently reported the remarkable crystal structure of one form of cholesterol monohydrate. The arrangement of the eight crystallographically independent molecules in the triclinic cell shows unusual local pseudo-symmetry. One pair of molecules is related to another by a non-crystallographic $b/2$ translation, while a third pair, oriented end-for-end relative to the first, is related to the fourth by a similar $a/2$ translation. Non-crystallographic twofold rotation symmetry relates members of the first and the third pairs. The overall structure is a stacking of bilayers of 33.9 Å total thickness. We report here that anhydrous cholesterol crystals are also triclinic, with eight independent molecules per cell. This structure offers additional insight into the packing of cholesterol molecules, and a comparison with the monohydrate structure.

Colourless lath-shaped crystals were obtained by cooling a saturated acetone solution of cholesterol. The cell dimensions are $a=14.172(7)$, $b=34.209(18)$, $c=10.481(5)$ Å, $\alpha=94.64(4)$, $\beta=90.67(4)$ and $\gamma=96.32(4)^\circ$, in agreement with values reported by Bernal *et al.*¹, and converted to a reduced cell³: $a=14.00$, $b=33.71$, $c=10.46$ Å, $\alpha=94.5^\circ$, $\beta=90.0^\circ$ and $\gamma=95.9^\circ$. Since cholesterol is an optically active compound, the space group of the triclinic crystals is $P1$. Each unit cell contains eight $C_{27}H_{46}O$ molecules; thus there are 224 non-hydrogen atoms per asymmetric unit. The calculated density is 1.021 g cm^{-3} ; the experimental value (floatation) is 1.03 g cm^{-3} . A total of 10,399 unique reflections were measured to the limit $2\theta(\text{CuK}\alpha)=100^\circ$ using a Syntex PI diffractometer with a graphite monochromator.

The structure was solved by a combination of vector search methods⁴ and direct methods⁵. Vectors representing the known geometry of the rigid 21-atom 3-hydroxy steroid group were used in Patterson-space orientation searches which gave several probable orientations of this group in the crystal. Vectors between steroid groups in these orientations were used in translation searches. In this way it was possible to position three oriented steroid groups in relation to each other in such a way that searches with the three sets of inter-group vectors gave a consistent result. This 63-atom partial structure was used to calculate input

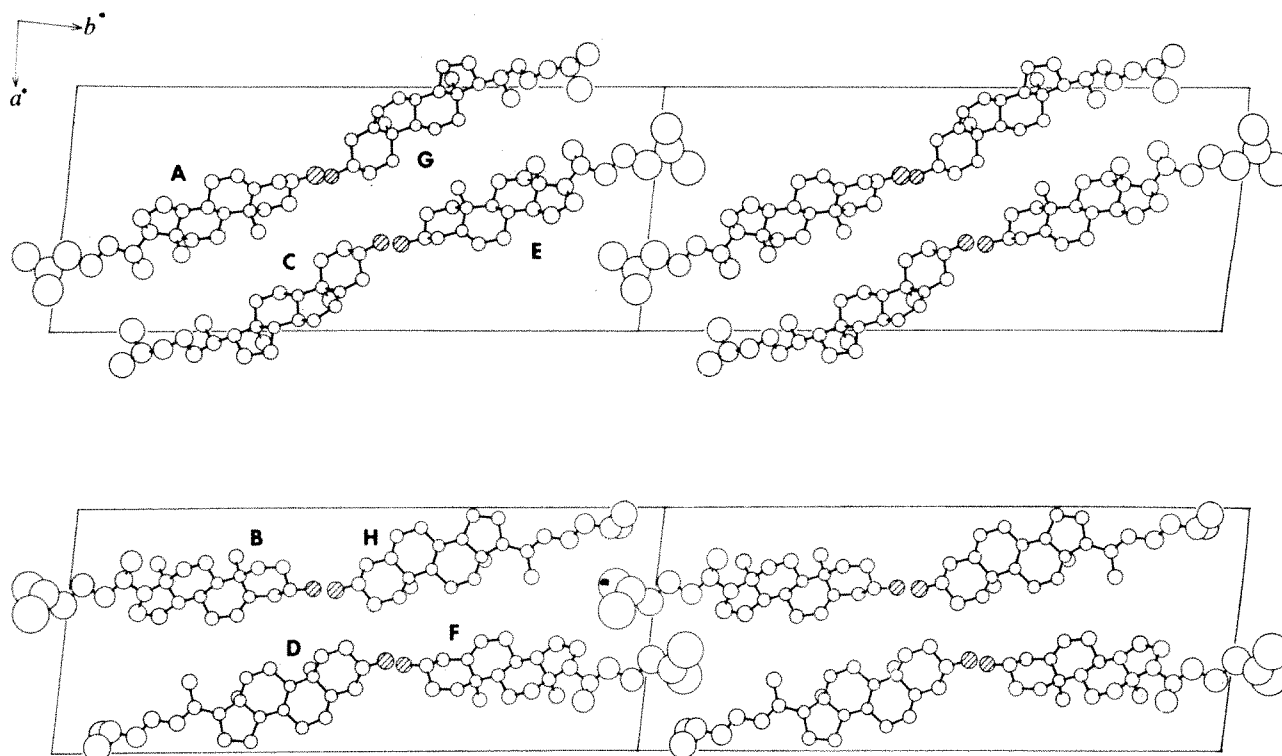


Fig. 1 The crystal structure of anhydrous cholesterol viewed along c (out of page). The upper figure is to be superposed on the lower one to give the complete structure. Two unit cells ($x = 0-1$; $y = 0-2$) are shown, displaying non-crystallographic twofold symmetry axes at $x \approx 0.5$; $y \approx 0.5, 1.0$ and 1.5 . Atoms are shown as 50% probability thermal motion spheres. Oxygen atoms shaded.

phases to the MULTAN programme³, modified to hold selected input phases fixed throughout the phase extension and refinement. The structure then emerged in a straightforward manner. About 80% of the hydrogen atoms were found in difference Fouriers, including those eight which are involved in hydrogen bonding. Refinement by block-diagonal least squares, with anisotropic C and O thermal parameters, gave $R=0.056$ for 8032 reflections with $I > 2.33\sigma(I)$.

There are no significant differences between the steroid skeletons of the eight molecules in this structure, the

average atom-atom misfit in superposing steroid skeletons being 0.07 Å. The conformation of the tail part of the cholesterol molecule shows considerable variation, however, as shown in Fig. 1, where molecules A and C represent two extreme cases in their C(17)–C(25) distances of 6.60 and 5.51 Å, respectively. This variability in the hydrocarbon chains is less pronounced in the monohydrate² where the chains are partially folded to an approximately equal degree.

The hydrogen bonding pattern (Fig. 2) consists of two independent chains of hydrogen bonds running parallel to

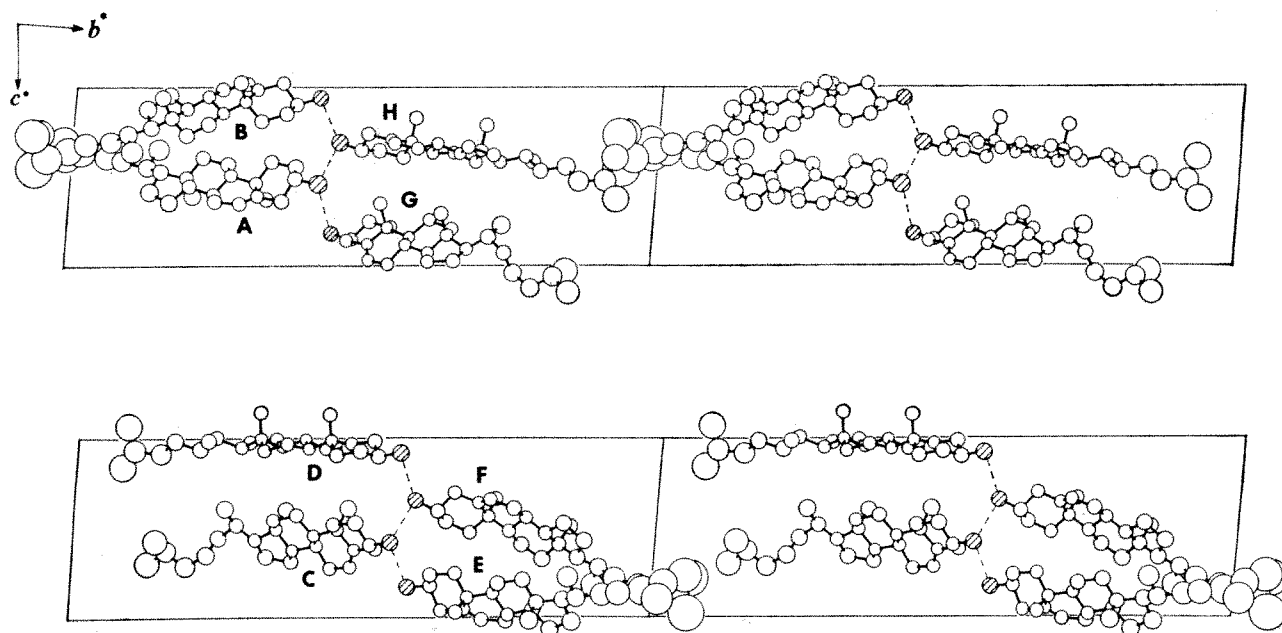


Fig. 2 The structure viewed along a (into page). Two unit cells ($y = 0-2$; $z = 0-1$) are shown, as in Fig. 1. The dashed lines indicate hydrogen bonding.

c with an average O...O distance of 2.87 Å. The hydrogen bond chains are staggered by ± 2.18 Å about a mean plane parallel to *ac*. This corrugated sheet of separated, parallel chains is to be compared to the pleated sheet of hydrogen bonds in the monohydrate.

The steroid planes of the eight molecules show no tendency towards parallel packing. The dihedral angles between least-squares planes of adjacent molecules range from 3° to 88°. The long molecular axes, while generally oriented along *b*, show considerable variation in direction. The angles between the least-squares lines calculated from any two molecules range from 4° to 36°. This is in contrast to the monohydrate, in which all the ring systems are approximately parallel and aligned.

Careful examination of the adjacent unit cells (Fig. 1) reveals an approximate local symmetry axis at $x=0.5$, $y=1.0$ along a direction making angles of 90.0, 94.5 and 0.7° with the crystal axes *a*, *b* and *c*, respectively. A 180°

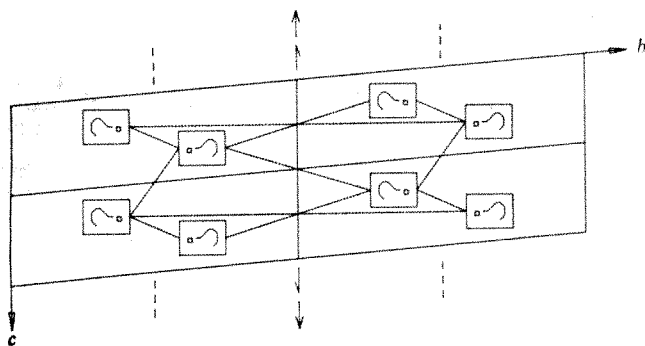


Fig. 3 Schematic representation of the non-crystallographic symmetry in four adjacent cells. The left- and right-leaning question marks represent, respectively, the molecular clusters ABCD and EFGH. Local symmetry elements shown are co-axial twofold rotation and screw axes at $y=1$ and a 'twofold screw axis' with alternating translational components of 0.25 and 0.75 *c* at $y=0.5$ and 1.5. This diagram applies at $x=0$ and 0.5.

rotation about this axis brings the leftmost molecules A, B, C and D into close coincidence with the molecules E, F, G and H of the adjacent (right hand) cell. The mean atom-atom misfit between the 112 atoms of the first four molecules, turned as a rigid group, and the second 112 atoms is only 0.24 Å. This fit is as good in the C(17) chain regions as it is in the steroid skeleton regions. The remaining molecules E, F, G, H of the left hand cell are related to A, B, C, D of the right hand cell by a pseudo-twofold screw axis which is coaxial with the pseudo-twofold rotation axis. Figure 3 illustrates in diagrammatic fashion the relationships among the molecules of four adjacent unit cells. Non-crystallographic symmetry axes can also be seen to exist at $x=0.5$, $y=0.5$ and 1.5 in Fig 3. These are 'screw axes' whose translation components alternate between (very nearly) $c/4$ and $3c/4$, instead of the usual one-half cell translation.

With the presence of this pseudo-symmetry, the non-crystallographic asymmetric unit contains four molecules. These four 'unique' molecules are A, B, C and D. As can be seen in Fig. 2, there is no hydrogen bonding between these molecules. It seems that this unique structural unit is held together primarily by hydrophobic interactions.

The number of 'unique' molecules, that is, molecules not related to each other by either local or strict symmetry,

is the same in the monohydrate² and the anhydrous form. The nature of the non-crystallographic symmetry in the two structures is entirely different; there is no non-crystallographic half-cell translation in the anhydrous structure. Yet, in each case, the non-crystallographic symmetry is obeyed locally to a remarkably high degree.

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Direct evidence for an interaction of β -adrenergic blockers with the 5-HT receptor

ADVANCES in neurotransmitter receptor identification and quantification by biochemical techniques involving radio-labelled analogues of putative neurotransmitters have led to the characterisation of a number of receptor systems in mammalian brain tissue¹; these include the catecholamines^{2,3}, 5-HT⁴, glycine⁵ and GABA⁶ receptors. The development of these techniques has provided a means of screening chemical compounds for their effects on different receptor systems⁷. The central nervous system effects of ($\pm$)-propranolol (Inderal, ICI) in animals are well documented^{8,9}. This drug has therapeutic benefit in man in anxiety¹⁰, and is also reported to have similar benefit in schizophrenia^{11,12}, essential tremor¹³ and drug dependence¹⁴. The origin of these effects is not understood but theories about its mode of action include β -adrenergic blockade¹⁵, membrane stabilisation⁸ and a 5-HT receptor blocking action^{16,17}. We report here that propranolol a stereospecific affinity for the 5-HT receptor from rat brain which is similar in magnitude to the putative 5-HT receptor blocking drug methylsergide.

Specific binding of ³H-5-HT was measured by the method of Bennett and Snyder⁴. The whole brain (minus the cerebellum) of a freshly killed Alderley Park rat (250-350 g) was rapidly removed and homogenised in 30 ml of ice-cold 0.32 M sucrose with five passes of an air-driven glass-Teflon homogeniser. After centrifugation at 1,000g for 10 min, the supernatant was subjected to 17,000g for 30 min to obtain a crude mitochondrial pellet. The pellet was lysed with 30 ml of ice-cold distilled water and briefly homogenised as before. The lysate was then spun at 10,000g for 20 min. The pellet from this spin, a bilayer with a soft, buffy upper layer was washed carefully to collect the upper layer and the resultant supernatant centrifuged at 48,000g for 20 min. The pellet was suspended in 6 ml of 75 mM Tris-HCl buffer (pH 7.4) containing 25 mM MgCl₂·6H₂O and 50 μ g ml⁻¹ of nialamide.

Incubations (15 or 16.5 min, 37 °C) were carried out in glass test tubes in a 1 ml final volume containing 75 mM Tris-HCl (pH 7.4), 25 mM MgCl₂·6H₂O, 5 nM ³H-5-HT (specific activity 14 Ci mmol⁻¹, Radiochemical Centre, Amersham), 640-840 μ g of protein, the appropriate concentration of the drug under test and in the presence or absence

of 10^{-6} M 5-HT. Incubations were terminated by filtration under reduced pressure through Whatman GF/C filter papers. The filtration procedure consisted of quenching the incubation with 4 ml of ice-cold buffer, rapid filtration, followed by two further washes with 4 ml of buffer. The complete washing procedure usually took less than 10 s. The residual radioactivity on the filter papers was counted at 31% efficiency in a PCS scintillant (Amersham-Searle). Protein was estimated by the method of Lowry *et al.*¹⁸. 'Nonspecific' binding, defined as the radioactivity left on the filter paper in the presence of 10^{-6} M 5-HT, was subtracted from total binding in each experiment to give 'specific' or receptor binding. Specific binding at 5 nM ³H-5HT was in the range 55–65% of total binding.

In accord with Bennett and Snyder⁴, we have shown that the specific portion of ³H-5-HT binding is rapid, reversible, saturable and of high affinity ($K_D = 11.7 \pm 2.5 \times 10^{-8}$ M, average of three experiments, $\pm$ s.e.m.). The concentration at which specific ³H-5-HT binding is inhibited by 50% (IC_{50}) by the two putative 5-HT blocking drugs, methysergide and cyproheptadine, are given in Table 1 and are close to the previously reported values⁴. Also shown in Table 1 are IC_{50} values for the 5-HT receptor of a number of β -

5-HT receptor reported here is in accord with these findings. The significance of these observations in relation to the effects of propranolol in the central nervous system of animals and man remains to be evaluated.

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Table 1 Effects of various drugs on ³H-5-HT binding to crude synaptic membranes

Drug	Half maximal inhibition of specific ³ H-5-HT binding (M)
5-HT	$1.7 \pm 0.3 \times 10^{-8}$
Methysergide	$2.8 \pm 0.3 \times 10^{-7}$
Cyproheptadine	$4.8 \pm 1.1 \times 10^{-8}$
(-)-Propranolol	$2.1 \pm 0.4 \times 10^{-7}$
(+)-Propranolol	$1.2 \pm 0.2 \times 10^{-8}$
(±)-Propranolol	$6.0 \pm 1.2 \times 10^{-7}$
(±)-Alprenolol	$3.0 \pm 0.5 \times 10^{-7}$
(±)-Oxprenolol	$4.3 \pm 0.8 \times 10^{-7}$
(±)-Pindolol	$7.9 \pm 0.8 \times 10^{-7}$
(±)-Practolol	$> 10^{-4}$
(±)-Atenolol	$> 10^{-4}$

³H-5-HT binding was assayed as described in the text. Results are means $\pm$ s.e.m. of triplicate determinations from at least three separate experiments using six different concentrations of the drugs indicated. The concentration of the drug giving half maximal inhibition of specific ³H-5-HT binding was determined by log probit analysis.

adrenergic blocking drugs. Propranolol has a substantial affinity for the 5-HT binding site, indeed (-)-propranolol is equipotent with the 5-HT blocking drug, methysergide, in displacing ³H-5-HT from its receptor. The interaction of propranolol with the 5-HT receptor is stereospecific as is evidenced by an approximately 58-fold lower affinity of the isomer (+)-propranolol, the racemate drug (±)-propranolol is about half as potent as the (-)-isomer alone. Other β -adrenergic blocking agents also have substantial affinity for the 5-HT receptor (Table) although β -blockers which are cardioselective *in vivo*, for example practolol and atenolol, have IC_{50} 's greater than 10^{-4} M.

Thus, we have demonstrated a stereospecific interaction of propranolol and other β -blockers with the 5-HT receptor, an action which was previously unknown for this class of drug. It seems that the affinity of β -blockers for the 5-HT receptor does not necessarily parallel their β -blocking potency in tissues outside the central nervous system since pindolol, an extremely potent β -blocker, is less potent in displacing ³H-5-HT than (-)-propranolol. Green and Grahame-Smith¹⁷ have suggested, on the basis of the blocking action of (-)-propranolol on a L-tryptophan induced hyperactivity syndrome in rats, that this drug inhibits central 5-HT function. The direct interaction of propranolol with

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Errata

In the article 'Possible detection of a radio event correlated with a γ -ray burst' by N. Mandolesi *et al.*, *Nature* **266**, 427–429, the legend to Fig. 2 should have read: Fig. 2a, Balloon horizon; b, Medicina horizon in equatorial coordinates . . .

In the article ' β -Endorphin *in vitro* inhibition of striatal dopamine release' by Horace H. Loh *et al.*, *Nature* **264**, 567–568, the last sentence beginning in the second column of p.567 should have read: Met⁵-enkephalin, on the other hand, did not produce a significant inhibition of tritium overflow in concentrations as high as 100 μ M. Naloxone, at an equimolar concentration, produced a significant blockade of the inhibition by morphine and β -endorphin, the blockade being more complete in the presence of morphine than in the presence of β -endorphin.

Corrigenda

In the article 'Pigmentation of the ladybird beetle *Coccinella septempunctata* by carotenoids not of plant origin' by G. Britton, W. J. S. Lockley, G. Harriman & T. W. Goodwin, *Nature* **266**, 49–50 the authors stated that phytoene, ζ -carotene and β -zeacarotene had not been detected before in any insect. Although no details were given of their characterisation, the detection of these carotenoids in certain butterflies has, however, been reported previously^{1–3}.

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In the article 'Nucleosynthesis and anomalous xenon and krypton in carbonaceous chondrites' by J. B. Blake and D. N. Schramm, *Nature* **263**, 787, the references on the following sentences with proper reference numbers should have read: Perhaps the explanation of the anomalous light isotopes lies in either the p-process^{8,10} or in fractionation⁷. If the light xenon component is due to the p-process then ¹³⁰Xe might be affected, which would change the normalisation⁹.

reviews

Birth of a Universe

John Barrow

The First Three Minutes. By Steven Weinberg. Pp. 188. (Basic Books: New York, March 1977; Andre Deutsch: London, September 1977.) \$8.95; £4.50.

DURING the twentieth century, cosmology has been transformed from metaphysics to physics, creating one of the most exciting arenas for scientific investigation. The fundamental laws of physics on the one hand and dramatic progress in observational astronomy on the other make it possible to reconstruct a general evolutionary picture of the Universe's past history with some certainty.

If one takes the present structure and reverses the sense of the observed expansion then extrapolation backwards in time reveals increasingly denser and hotter cosmic epochs. The times of important event strata can be established in the manner of geology until eventually a 'big bang' is envisaged 10,000 Myr ago. This hot scenario for cosmogeny is based on the existence of a cosmic background radiation field, predicted by Gamow in 1948, and detected at microwave-lengths by Penzias and Wilson in 1964. The photons of this radiation background are now unanimously interpreted as a vestigial remnant of the extreme conditions prevailing in the primordial fireball itself, when matter was completely ionised and the Universe a high energy physicist's paradise.

The increasing temperature as we move further into the past towards the 'bang' ensures that cosmology can provide a remarkable theoretical laboratory for particle physics. Of particular importance has been the realisation that during the first few minutes of expansion from the 'bang', physical conditions were expedient for the fusion of light nuclei having distinctive abundances. The fossilised abundances of helium and deuterium have been detected in precisely the range predicted by the simplest models and reassure us that a view into the early opaque phase of the Universe's life is possible by indirect means. The contingency of thermodynamic equilibrium in the first few seconds washes away all the uncertainties surrounding the initial state and enables laboratory physics to develop a fairly complete description of events.

Steven Weinberg has again very successfully turned his attention from the world of microphysics, where his contributions are well known, to cosmology. *The First Three Minutes* is a clear and challenging description of the Universe's early evolution suitable for the informed layman and practising scientist alike. The author has vividly explained the essential concepts and physical processes involved, without recourse to mathematics or the details of general relativity, by introducing the necessary physical ideas as his story unfolds. One of the remarkable features of the book is the manner in which the author has maintained the rigour of his physical argument throughout, never sacrificing logical continuity in the interests of speed or oversimplification.

The first three chapters are partially historical in content, describing the discoveries of the universal expansion and microwave background radiation. Unfortunately, no explanation of the crushing blow dealt to the steady-state theory by the latter observations is provided. Such a description is essential in a work intended for the non-specialist, who often believes this theory still to be in strong competition with the 'big bang' cosmologies.

In the fourth chapter the physics of black-body radiation is described with great clarity, together with the conserved quantities necessary to specify the microstructure of the Universe uniquely. This careful build-up prepares the reader for the key chapter in which the phenomena peculiar to the first three minutes of expansion from the origin are expounded frame by frame. The final chapter is an ex-

cursion into the history of science in an effort to discover the reasons for scientists' procrastination in establishing this picture when the necessary observational and theoretical ingredients were apparently available.

Criticisms of the book are minor. Perhaps a little more space could have been allocated to the first three minutes rather than the past fifty years. The opportunity could then have been taken to mention Hawking's exciting work on primordial black holes or problems surrounding the existence of a singularity in spacetime. Some more clear diagrams displaying the evolutionary phases considered and their generic phenomena would also have been welcome landmarks to establish the overall panorama more vividly. Finally, a little more space might have been devoted to the conceptual problems associated with the standard model; these latter are of great intrinsic interest and motivate much current work in cosmology. Weinberg more than atones, however, for these failings with many fascinating digressions into points of basic physics and by a lucidity that makes for compelling reading. The provision of a glossary of technical terms, detailed index and bibliography are further attractive features of what will surely become a landmark in the exposition of the subject.

This book can be strongly recommended to all as the place to begin or reinforce their education in 'pre-historic' cosmology. □

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Nerve, synapse and muscle

Electrobiology of Nerve, Synapse and Muscle. Edited by J. P. Reuben, D. P. Purpura, M. V. L. Bennett and E. R. Kandel. (Raven: New York, 1976.) \$30.

WRITTEN by his students and associates, this *Festschrift* volume in honour of Harry Grundfest contains a collection of recent studies on nerve, synapse and muscle. As might be expected from Grundfest's own interests, this volume ranges widely and provides fascinating glimpses of the ways in which an im-

portant area of biology has grown and developed. The chapters are uniformly interesting and no electrobiologist could pick up this book and fail to learn something new. Cell physiologists around the world will be delighted that Harry Grundfest's contribution has been honoured in this way.

P. F. Baker

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Mammalian cell membranes

Mammalian Cell Membranes. Vol. 2: The Diversity of Membranes. Vol. 3: Surface Membranes of Specific Cell Types. Edited by G. A. Jamieson and D. M. Robinson. (Butterworths: London and Boston, Massachusetts, 1977.) £18 each volume.

THESE two volumes continue an extensive coverage of the structure and function of cell membranes under the general editorship of G. A. Jamieson and D. M. Robinson. As in the first volume of the series, the editors adopt a relaxed attitude towards the form and content of each contribution, with the inevitable results of an uneven mixture of styles and a considerable amount of overlap in content. Nevertheless, a considerable amount of enjoyment is to be had from these books.

In the opening chapter of volume 2, Emmelot provides an excellent short account of the composition and organisation of the plasma membrane. His discussion of the interactions of the plasma membrane with microtubules and microfilaments, one of the growth areas in present-day membrane research, is particularly useful and includes a reminder that some of the initial effects of various drugs disrupting cytoplasmic contractile elements may be directly on the plasma membrane. Kramer and his colleagues then present a personal and very interesting account of membrane flow, the migration of components from the membranes of the endoplasmic reticulum to other membranes including the plasma membrane.

These two rather general reviews are followed by short chapters concerned, respectively, with membranes of the Golgi apparatus (Favard), mitochondria (Capaldi), lysosomes (Wattiaux), microbodies or peroxisomes (Tolbert and Donaldson) and nuclei (Fry). The volume is rounded off with two somewhat dated accounts of myelin and basement membranes having no full reference later than 1973, a serious drawback in these rapidly expanding fields, in which new membrane components are turning up regularly to transform previous views of membrane organisation. It is surprising too that the primary sequence of the basic protein of myelin, which was established several years ago, is nowhere discussed, particularly in connection with its encephalitogenic properties.

The most complete analysis of glycoprotein structure is in the first chapter in volume 3 by Segrest, which is particularly valuable in presenting his current views on the molecular topography of the non-polar domain of this glycoprotein. There is interesting speculation on the state of self-association of glycoprotein within the membrane and the possibilities of these aggregates in facilitating transport. One of the editors (Jamieson) provides an authoritative summary of platelet ultrastructure, perhaps the clearest case for intimate inter-relationships of the plasma membrane with underlying microtubules in a cellular function. Jamieson also summarises the intriguing evidence for collagen-platelet interactions being mediated through surface located glycosyl transferase. As Jamieson points out, some of the disagreement in this area may be due to an unwarranted extension of his original theory, concerned with simple adhesion of platelets on to collagen, to collagen-induced platelet aggregation. The membrane systems of lymphocytes and purification of their antigenic markers are then reviewed, followed by summaries of the extensive literature on tumour cells including an excellent fresh appraisal of the complex "transformation phenotype" by Paster-

nak. Volume 3 closes with three descriptive essays, respectively on the sarcoplasmic reticulum of muscle, composition and structure of excitable nerve membranes and the interactions of sperm and egg membranes in fertilization, and finally a detailed account by De Luca of vitamin A biochemistry and effects on epithelial cell membranes. The role of retinol compounds in transglycosylation is well covered.

To summarise, the series on *Mammalian Cell Membranes* is shaping up as a useful collection of articles which, despite considerable dispersal and overlap between the various volumes and rather indifferent indexing, serves a useful purpose as a source of the recent membrane literature. In one or two cases the author does more, illuminating as well as cataloguing the field. One further point: although the line drawings are in general excellent the reproduction of original photographs and electron micrographs is usually poor. In volume 2, which relies heavily on such reproductions, this is a serious drawback and is always a nuisance since often the pictures illustrate a crucial argument.

R. C. Hughes

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Predator-prey relationships

The Ethology of Predation. By E. Curio. Pp. x+250. (Springer: Berlin and New York, 1976.) DM72; \$29.60.

THIS is a thorough and well-presented review of a wide range of literature up to and including 1973 on the general subject of predator-prey relationships, primarily from a behavioural point of view. It will serve as a valuable reference work for many years to come. Although the main bulk of the book is concerned with behavioural aspects of predation (using the term in a very broad sense), dealing with questions of how animals detect, search for and capture food, there are brief excursions into physiological and ecological aspects of the subject. These peripheral, but important areas are dealt with in less detail than the pure behaviour, and one feels that the author is less happy with them. For example, among the ecological aspects, the discussion of the functional response contains several minor errors, such as the assertion that a type 3 response only occurs when there are at least two alternative prey types. Similarly the summary of optimal foraging theory on pages 70-80, heralded on the book cover as a link

with modern ecology, is confusing and somewhat inaccurate, and the author does not emphasise that the theory he presents at this stage applies equally to problems discussed in other parts of the book. Much of the literature on optimal foraging has appeared since 1973, so Curio's account is already dated.

Curio is, however, encyclopaedic, accurate, and critical in reviewing the more traditional ethological literature. For example, both his discussion of prey selection and the accounts of specialised hunting techniques are excellent sources of information. In the latter sections, the literature survey covers an impressive range of examples, from African natives stalking their prey under the disguise of a stuffed hornbill's head, to Green Herons hunting for fish by dropping pellets of commercial fish food into a pool at the Miami Seaquarium. My only criticism of these behavioural chapters is that the material is presented as a series of rather separate sections without a great deal of attempt to unify them. This makes this useful book more like a work of reference rather than an introduction to the subject.

John Krebs

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Chemisorption on evaporated films

Chemisorption: An Experimental Approach. By G. Wedler. Translated by Derek Klemperer. Pp. vi+250. (Butterworths; London and Boston, Massachusetts, 1976.) £12.

'*Quot homines, tot sententiae*', that venerable Latin tag, is particularly applicable to chemisorption. Since the recent invasion of the subject by physicists and mathematicians it has become so vast, that a full account of the simpler gases on metals alone would require a separate volume for each metal, and a chapter for each lattice plane. Therefore, it is particularly sensible that Professor Wedler, in this broad conspectus of methods, should tend to emphasise the approaches taken by his teacher, R. Suhrmann and himself, to chemisorption on evaporated metal films. This does mean, however, that a proper balance is hardly given to some of the earlier work—for example, Otto Beeck's film work is mentioned but no reference is made to J. K. Roberts' even more crucial studies on tungsten wires, which many regard as a turning point in the subject.

The index reveals no reference to accommodation coefficients or heats of adsorption on wires. Other important more modern techniques are also omitted, such as the Mossbauer effect, and neutron diffraction. The new PhD student starting up in the field would still be well advised to read the Hayward and Trapnell book (*Chemisorption*, Butterworths; London, 1964) to get the scholarly background to

the modern methods described here. But having done this, he or she will find Professor Wedler's book makes easy reading and gives a fairly wide coverage, with 744 references up to 1974. (Here a word of praise should be given to Dr Klemperer for his translation and updating of the German (1970) version.) In addition, it ends up with a short discussion of selected data on Ni/H₂ and Ni/CO, and brings Trapnell's well known Table up to date; it does, however, exclude the hydrocarbons.

Third-year undergraduates doing surface chemistry and research students will find some sections useful, but would be advised to read up nuclear and electron spin resonance elsewhere. How can one possibly explain electron spin resonance in two pages? This is a subject where *g*-factor anisotropies and hyperfine interactions (no mention of these) have contributed vital information covering the adsorbed species on oxides such as SiO₂ and MgO, and a little more effort could have given this topic its proper place in the whole picture.

Experienced workers will want to refer to it for the areas of special expertise of its author, such as electrical and heat measurements on evaporated metal films. Indeed, a better title for the book would surely have been *Chemisorption on Metals*, since there are very few references to metal oxides, or other systems apart from metals. With these qualifications, its purchase can be recommended to science libraries with an interest in the topic concerned.

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Our living planet

Earth, The Living Planet. By Michael J. Bradshaw. Pp. 302. (Hodder and Stoughton: London, Sydney, Auckland and Toronto, 1977.) Paperback £4.95.

THIS volume is intended as "a reader for sixth-form [advanced school] and college courses in physical geography and environmental science", and is a companion to *The Earth's Changing Surface*, about the solid Earth. *Earth, the Living Planet* divides almost equally between a description of the atmosphere, oceans, weather systems and climates of the Earth (the first half of the book) and a description of the kind of life that has evolved and adapted to those conditions. The large (A4) format, generous use of diagrams and a section of colour illustrations help to provide an attractive overall presentation, and make it easy for the reader to dip into the book for selected highlights. In a few places the need for access to the companion volume handicaps the reader who has only bought

this one of the pair; but by and large the book stands well on its own as an introduction to both the physics and the lifeforms of the biosphere.

There are, however, some deficiencies in the presentation, mostly due to over-compression of the material into a limited space. The discussion of weather modification, for example, could well have been developed further, and I was disappointed to find that the chapter "Climates of the Past—and Future?" made no mention of the Milankovitch model of Ice Ages, which in its modern form has now become widely accepted as at least a partial explanation of climatic changes on timescales of a few thousand to several tens of thousand years. By and large, the book provides an excellent introduction to our living planet, at a remarkably reasonable price, and will be useful not only as an introduction for new students but as a refresher for those whose student days are more remote.

John Gribbin

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Exploration geophysics

Applied Geophysics. By W. M. Telford, L. P. Geldart, R. E. Sheriff and D. A. Keys. Pp. xvii+860. (Cambridge University: Cambridge, London and New York, 1977.) Hardback £26; paperback £9.75.

THIS textbook of exploration geophysics is intended for practitioners and graduate students, replacing the classic works such as those of Heiland and Jakosky which are now outdated. It stems from another classic, that of Eve and Keyes published twenty years ago, but is far more than a revision of that book, reflecting two decades of particularly rapid advances in both instrumentation and interpretation. The treatment is comprehensive both in coverage of all the important geophysical techniques (developments in remote sensing seem to be the only omission) and in dealing with the practical, theoretical, geological and physical aspects of geophysics in a balanced way. The text is supplemented by problems, mostly using real data, some of which are drawn together in the last chapter to provide examples of integrated interpretation, usually in a mining context.

Over a quarter of the book is concerned with the seismic method. This section has been most successfully updated, and includes sections on the essentials of digital data processing and on the geological interpretation of seismic reflection sections. The refraction method is treated only very briefly but with a clarity which is a feature of the whole book.

The chapters on potential field and electrical methods are rather less satisfactory, giving undue emphasis to instruments, interpretation methods and units now best consigned to the museum: for example, several obsolete methods for the interpretation of resistivity soundings could well have been given as references only, saving space for the 'resistivity transform' approach to this problem. These chapters also assume some knowledge of vector calculus, but the rest of the book should present no mathematical difficulties even to readers trained primarily as geologists.

It is still true that no single textbook is sufficient for the education of a graduate geophysicist, but this one is certainly necessary for reading and reference. At little more than a penny a page in paperback, it has the advantage of being within his means.

R. F. King

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Martian geology

The Geology of Mars. By T. A. Mutch, R. E. Arvidson, J. W. Head, K. L. Jones and R. S. Saunders. Pp. ix+400. (Princeton University: Princeton, New Jersey, 1977.) £28.40; \$35.

WRITING a book about our planetary companions in the Solar System is, and has been for over ten years, a hazardous business. The rate of exploration has been such that inevitably parts of such a book are out-of-date even before it arrives on the bookstands; this is because new, important and often revolutionary information is returned from another exciting space mission. Despite this, and despite the fact that we are in the middle of the highly successful Viking mission to Mars, Thomas Mutch and his colleagues have produced an extremely timely book on the geology of Mars. Essentially, it summarises our knowledge of this planet up to the time of Viking, although it does include pictures and information from the early part of the Viking mission slipped into the book at the last moment. The book is a valuable contribution to planetary studies because it provides (as did Glasstone's *The Book of Mars* before Mariners 6, 7 and 9) an up-to-date summary of our knowledge of this fascinating planet up to the time of Viking—a useful time to stop and take stock of the situation.

The book opens with two chapters describing early telescopic observations and the history of spacecraft exploration of Mars. This is followed in the third chapter by a summary of the major physiographical provinces of Mars and a discussion of how each of the landforms characteristic of different parts of Mars may have been formed. The main bulk of the book consists of individual chapters on surface processes including impact cratering, volcanism and the action of wind and water, all of which have played a part in producing the martian surface as we see it today. There are also discussions of geophysics, structure and geological history of Mars.

Geologists not involved in planetary studies have often been disappointed with new discoveries from both the Moon and Mercury because so much of the geology of these planets is dominated by impact cratering, a geological process of interest to only a few specialists. This is not true of Mars as, although the ancient southern hemisphere of Mars is dominated by impact cratering, the northern hemisphere and poles show abundant

evidence of other processes more familiar to the terrestrial geologist. Large areas have been modified by wind erosion and deposition, and the desert geomorphologist cannot fail to be impressed by the extensive dune fields exposed on the martian surface. Winding channels are indicative of water erosion and the areas around the polar caps show thick sequences of layered rocks. Although volcanism probably played an important part in the evolution of all terrestrial planets, it is only on Mars and Earth that we have seen such a variety of large constructs of high topographical relief. Extensive patterns of rifts and faults show that the tectonic history of Mars has been quite unlike that of the Earth and an understanding of this is essential to developing a general theory for planetary tectonics.

Molecular potential energy surfaces

Symmetry Rules for Chemical Reactions: Orbital Topology and Elementary Processes. By R. G. Pearson. Pp. ix+548. (Wiley-Interscience, New York and London, 1976.) \$31; £17.50.

THE title of this work is not altogether successful in describing the contents. I do not venture to suggest a better one; but Professor Pearson's concern is with the nature of molecular potential energy surfaces generally, and with their influence on the shapes of molecules as well as on the courses of reactions. His thesis rests on two main principles. The first, due principally to Bader, although certainly used earlier, is that a distortion of a particular symmetry is favoured by the presence of a low-lying electronically excited state of a related symmetry. (Perhaps this should be called the 'Bader effect' since the usual 'second-order Jahn-Teller effect' is a clumsy and inaccurate term.) The distortion may lead a molecule from one postulated structure to another, in which case one obtains an understanding of the shapes of molecules. Walsh's rules are usefully approached in this way. Alternatively, the distortion may initiate a reaction, in which case one obtains an understanding of the relative ease of alternative reaction paths.

The second principle is that of "orbital correlation" or "conservation of orbital symmetry": the symmetry species of the molecular orbitals in reactants and products must match. If they do not, then at least one bonding orbital in the reactants correlates with an anti-bonding orbital in the products,

The authors of this book do an excellent job of summarising and discussing critically our knowledge of all these subjects. They also briefly compare Mars with the other planets, including Earth, showing how all the solid planets in the Solar System are important field areas for Earth-bound geologists.

This book will not only be a useful reference book for planetary geologists but provides an easily readable and well illustrated review for the non-specialist. For a student it is an important text, although sadly the price is high (especially the sterling equivalent); and students may have to rely on library copies. **J. E. Guest**

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which in turn implies a high activation energy. In the application of these principles, a third is used: the predicted behaviour depends on the "orbital topology"—the nodal structure of the molecular orbitals—which is not significantly modified even if the symmetry is broken by irrelevant changes to the molecule. Consequently symmetry-based methods may often be used where no formal symmetry is present.

Professor Pearson expounds these principles with admirable clarity and numerous examples. The Jahn-Teller selection rule is incorrectly stated (the correct version is subsequently used, but with no explanation of the notation for the symmetrised square) and the account of the first-order Jahn-Teller effect contains some very dubious remarks. Fortunately, the first-order Jahn-Teller effect is of only peripheral importance in the later development. A more general criticism is that the magnitudes of the effects cited are not always shown to be sufficient to account quantitatively for the observations. For example, the dissociation energy of ozone is 1 eV, whereas that of sulphur dioxide is 5.5 eV; this is explained by the fact that the relevant excited state in ozone is at 4.7 eV, whereas the corresponding state in sulphur dioxide is at 5.3 eV. The difference is in the right direction but the numerical values are unconvincing.

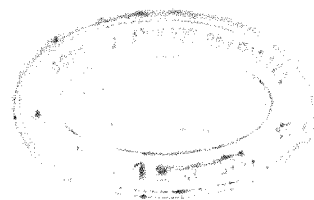
For the most part, however, Professor Pearson approaches his subject carefully, critically and thoroughly. This is a stimulating and valuable book, and an important contribution to a rapidly developing field of research.

A. J. Stone

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nature

26 May 1977



Risk adviser: mission impossible?

AN idea that has received wide publicity in the past week or two has been the concept of an independent community risk advisory service. Professor Gordon Atherley, of the University of Aston in Birmingham, presented the idea to the Council for Science and Society's meeting on acceptable risk and proposed that the council should take some initiatives itself to establish such a service. For its part, the council, in its forthcoming report on the acceptability of risks, commends the idea—'only in such a way can there develop an involvement of people experiencing risks, which is so necessary for fair decisions and effective control'.

There are many attractions to the idea. Professor Atherley envisages the scheme being staffed by a small band of volunteers with a scientific training who would agree to attend 'clinics' regularly in local communities to listen to and advise on problems connected with risk. The council suggests a full-time paid adviser, using part-time help. The concept would certainly be moving with the social tide, which is undoubtedly flowing away from monolithic centralised organisations towards local, more informal bodies. And analogies can be found, for instance in community law centres set up to help those who might be overawed by the legal establishment or its fees. The intention is that in the main the adviser would act as a source of information, maybe doing a little monitoring or research himself or herself. The proposal is clearly thoroughly worthy, in keeping with the council's previous institutional suggestions. But the question is, is it workable?

It is certainly going to tread on a few toes. The relationship of 'risk' to pollution, infectious diseases, safety at work, hygiene, public order, nuclear safety, fire and explosive hazards and road and rail safety is going to be hard to define. In all of these there are already deeply entrenched interests working at local or national level; it is certainly not clear that such interests, many of which have an excellent if unpublicised track record, will take at all kindly to a new figure in the community. This need be no bad thing if the community risk adviser were able to pursue an independent course of research, but this is most unlikely—in many matters the adviser will have to depend on these very organisations for data and even interpretation. Can this be done without the generation of ill-will or corruption? Of course a risk adviser would

ideally be on the lookout for new risks for which no control mechanism yet existed and might, for instance, have some success in the field of noise; but in most other fields he or she might need almost extra-sensory perception to foresee the extraordinary ways in which risk appear.

However attractive the idea of spreading information about risks around the community in the same way that a legal service answers questions about the law and a consumer bureau provides ratings of relative value for money, there is a danger that the function of a specifically risk-oriented agency would be misunderstood as a place where answers would be given. And then, of course, the community would quickly turn on an adviser whose subjunctive-ridden replies had been misunderstood as authoritative pronouncements and proved wrong by later events. Would this make for a fulfilling job for a community risk adviser? It is perhaps in questions about the calibre of a person who would do such a job that we have the most serious reservations. Unpaid volunteers, says Professor Atherley; £3,500 per year, says the council—it is difficult to be serious about a scheme relying so much on voluntary involvement or modest salaries. The community risk adviser would have to be such a paragon of virtue, so widely knowledgeable, so unswayed by pressure groups, so diplomatic in dealing with toe-trodden agencies, so tough in living with decisions, that it is doubtful that many likely candidates exist.

That a community risk adviser may not be the way to go does not, however, close the issue. The social tide is still flowing and the public is bound to want to know more and not less about hazards and nuisances. An immense amount of expertise already exists in the existing agencies and it seems desirable that before we set up yet another we should look very carefully at how well the present agencies present themselves to the public. It is as yet by no means clear whom one should write to, phone or go and see when worried about potential or actual hazards. More informative telephone directories, advertising on television and in newspapers would all help to encourage the public to take a less Kafkaesque view of governmental agencies. If in a few years there was no growing sense of public participation in coming to terms with risks, then it might be time to try and set up a risk advisory service. But let the present agencies show their paces first. □

International science (1): NATO

Plain-clothes science

Chris Sherwell, recently at NATO,
looks at the alliance's science programme

WHAT is NATO? To most people it is simply the western alliance, the collection of fifteen nations formed after the Second World War for the purposes of mutual defence. Others see it as much more. For instance, when Jimmy Carter delivered his keynote address at the recent NATO summit in London, he saw the alliance as political and economic as well as military—which of course it is.

Indeed, NATO has always been these things and more. So broad is it that, to the amazement of many western scientists, it stretches as far as a civilian science programme. In the vast concrete labyrinth of buildings and corridors that are NATO's European headquarters in Brussels, there is a handful of offices occupied by a friendly group of what in other continental circles are usually called 'committed Europeans' because of their internationalism. There are just over half a dozen of them, and they discharge responsibilities created almost a decade after the North Atlantic Treaty was signed in 1949.

That treaty, which is most explicit about the threat of war and the resolution of disputes, contains in Article 2 vaguely worded sentiments about a better understanding of the principles upon which free institutions are founded. At the end of 1956 a committee of three foreign ministers—Lester Pearson (Canada), Gaetano Martino (Italy) and Halvard Lange (Norway)—produced a report on non-military cooperation. Like the devil and sin, they were for it; in particular they argued that science and technology was "one area of special importance to the Atlantic community".

From this and further suggestions by these so-called 'Three Wise Men' for recruiting and fostering contact between scientists, was born a Task Force on Scientific and Technical Cooperation. That was in July 1957. Then, fortuitously, came the single most important event to ensure the future of a NATO involvement in civilian science: the launching of the first Soviet Sputnik in October 1957. Two months later the Task Force reported its findings to the heads of government. Its recommendations were predictable.

The future of the West, the Task Force proclaimed, was dependent to an ever-increasing degree on the rate at which science and technology advanced. It was therefore in NATO's

interest to do all it could "to bring about the general conditions which will accelerate the pace of scientific progress"; deficiencies in science and technology should not be allowed to hamper the effort to strengthen NATO members' economies and defences.

Committee plus adviser

That was the reasoning, and it was accepted. Institutionally it produced NATO's Science Committee and a Science Adviser to the NATO Secretary General who was soon called Assistant Secretary General for Scientific Affairs. He is now Professor M. Nimet Özdas from Turkey, a mechanical engineer by training; he has occupied the position since 1973 and is the eighth holder of the post. Among other things, he chairs the Science Committee, on which sits one representative for each country (the SRC head, Sir Sam Edwards, represents Britain, for example); it met for the first time in March 1958 and, unsurprisingly, there are plans afoot for a Commemoration Conference next spring to mark NATO's 20 years in the business. What form that will take remains unclear as yet. What business NATO has been in is available for anyone to see.

One business it is not in with its science programme is defence-related research, save in a most indirect and thus unimportant sense. That activity falls under a separate division of NATO altogether. Because of his position Özdas is aware of work being done, but it has little to do with the nuts and bolts of his science programme. Özdas does say the security of the alliance is important as a purpose behind NATO's science programme, but then he could hardly be expected to say otherwise. The programme's direct impact is confined largely to the scientific community it seeks to help. Indeed, a cursory examination of the work supported and the cushioned impact it has outside scientific circles suggests that NATO's involvement in science is as benign as that of practically any other funding agency.

If there is any keynote, it is collaboration. Next to publication, collaboration is seen as the essential ingredient for the progress in modern research that is NATO's expressed concern. But NATO is anxious that it should do things which aren't already done and that this should happen

without at the same time adulterating in any way the established standards that ensure quality work.

It is admitted that the latter has not always been achieved—that in earlier days mistakes were made and NATO could have acquired a reputation for being a 'soft touch'. But its peer review system has been tightened up in recent years. If charmed circles of researchers who know the system extract money more easily than others, it isn't easy to prove. The system is open to anyone, and the criteria are clear. Where there is abuse, the only sanction at NATO's disposal is to refuse money the next time round.

Altogether, six fragments make up NATO's efforts to stimulate scientific cooperation. A Science Fellowships Programme, coordinated by Bulent Bayraktar, who is from Turkey, consumes the bulk of the Science Committee budget of some \$9 million. In this field Özdas believes that NATO is unique. No other organisation, international or otherwise, is funding the international exchange and foreign training of young postgraduate and postdoctoral students on such a scale (500–600 fellowships a year).

The focus of the second biggest consumer of funds, the Advanced Study Institutes (ASI) Programme, is higher up the academic ladder. Again emphasising international aspects and seeking to do something unique, the programme aims to provide high-level tutorials and seminars on selected topics, usually of an inter-disciplinary character, on the basis that such matters might not otherwise be covered and the people involved in them might not otherwise come together.

NATO funds some 50–60 applicants a year who are themselves responsible for organising the ASI. Each ASI usually lasts up to a couple of weeks and can involve as many as 30 lecturers and 100 participants. The coordinator of the programme from Brussels, Tilo Kester, a German, receives applications each year running into hundreds which must be refereed after initial screening. The \$30,000 or so that each ASI costs goes entirely on travel and living expenses; the direct benefit gained by the organiser is chiefly in the form of a publication.

International collaboration between scientists is further encouraged by the Research Grants Programme. International collaboration, in fact, is the *sine qua non* for participation in the programme, which offers financial aid to joint projects that involve laboratories and institutes in different countries. What NATO tries to do is fill the gaps left in respect of travel by national grants.

Some 75% of the money disbursed goes on living and travel expenses, and

the awards, which are also determined through peer review, are now of the order of \$4,000 where they were once up in the \$30,000 bracket. Demand has increased in recent years, and the programme's coordinator, Mario di Lullo from Italy, insists that the prospect of publishing either one or several papers is not a passport to a successful application, even though publications are expected to have a NATO credit.

Two relatively minor activities funded out of the Science Committee budget are the Senior Scientists Programme, which awards a small number of senior fellowships and visiting professorships each year, and the Science Committee Conference Programme, which, unlike other programmes, is organised directly from Brussels. Less than a dozen of these special conferences on particular topics have been held.

The sixth fragment of NATO's science activity is intentionally the least general but it is no less complex to administer. It breaks down into six parts. Known collectively as Special Science Programmes, they complement the other programmes and are designed to ensure coverage of specific topics. These include marine science, eco-science, air-sea interaction (a meteorology programme ended in 1972), materials science, systems science and behavioural science.

Seventh fragment

Özdas has another important position. This stems from a decision taken at NATO's twentieth anniversary meeting of heads of government in 1969 to create the Committee on the Challenges of Modern Society (CCMS). Özdas chairs this committee, which operates in an interesting way to avoid expensive, secret and centralised direction.

The CCMS has no budget. If any study is to be conducted, there has to be a pilot country or countries to shoulder the responsibility, probably with other countries as 'co-pilots'. They provide the funding, and they do the study. It is carried out on the basis of information readily available, with a short time horizon in mind. And for the most part it is conducted in an open fashion with participation not just by interested organisations within the alliance countries but also by countries outside the alliance—including the USSR and East Europe.

The CCMS proposes, the NATO Council approves. Eight studies have been completed and another eleven are under way. Topics include pollution, disaster assistance, health care, energy and so on.

Özdas is assisted in the discharge of his duties by Dr Philip Hemily, an American. A man sincerely concerned to know current trends in thinking

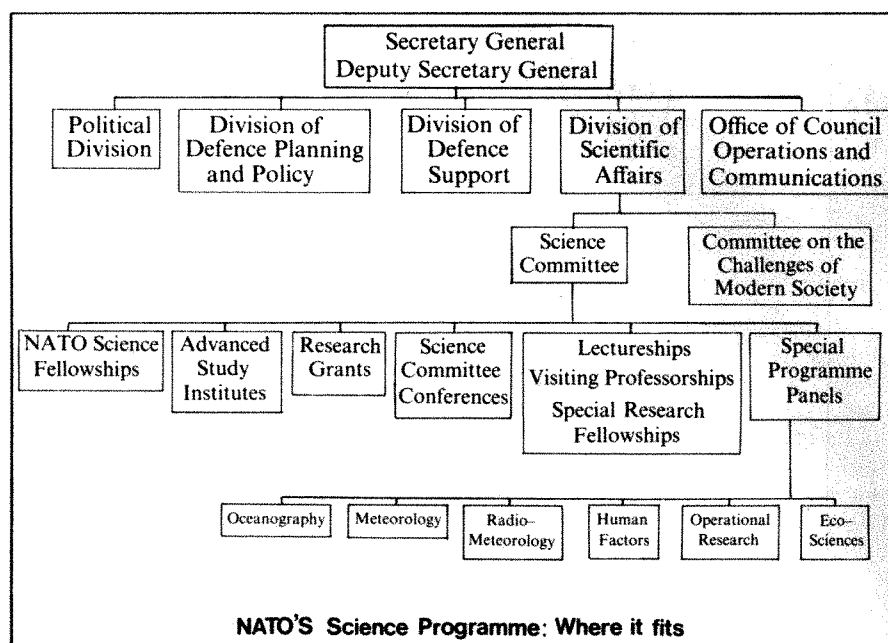


Table 1 NATO Science Committee budget (Belgian francs $\times 10^6$)¹

	1972	1973	1974	1975	1976	1977	% breakdown
Fellowships	135	137.7	144.0	148.0	160.0	172.0	54.5
Advanced Study Institutes	46.5	50.0	60.0	55.0	61.0	72.0	20.8
Research Grants	30.5	34.5	35.0	36.0	39.0	45.0	13.3
Programme Development Fund	29.5	33.0	28.0	34.0	30.0	31.0	11.4
Total	241.5	255.2	267.0	273.0	290.0	320.0	—

¹£1 = BF 62.4, \$1 = BF 36.3; all figures in current terms.

Table 2 Distribution of funds (%)

	Overall budget	Science fellowships (1960-72)	Research grants (1960-72)
United States	24.20	12.63	10.8
United Kingdom	19.50	13.96	17.2
France	17.10	11.98	6.2
West Germany	16.10	13.43	5.8
Italy	5.96	15.00	18.0
Canada	5.80	4.90	3.9
Belgium	2.86	2.74	3.8
Netherlands	2.85	2.62	3.3
Denmark	1.65	1.60	2.0
Turkey	1.65	9.65	11.0
Norway	1.15	1.57	3.7
Portugal	0.65	4.40	5.1
Greece	0.39	4.20	8.0
Luxembourg	0.09	0.26	0.0
Iceland	0.05	0.25	1.5
NATO (Adm Exp)	—	0.81	—

about contemporary problems, he is realistic enough to know the limits of NATO's capacity to help solve them. Of both the virtues and pitfalls of NATO's involvement in science he and his colleagues are only too aware. But they remain committed to the ideals which inspire scientific research, and see a role for NATO.

Thus, asked the obvious question, "Why NATO?", their immediate response is equally obvious: "Why not?" Pressed further, they steer clear of

arguments about common defence and security, preferring to fall back on another point: that there are no apparent alternatives available. No other international body is apparently working to oil the wheels of scientific research by providing finance to encourage international collaboration. That becomes their most positive argument: in the jargon of the men at NATO, they are 'action oriented'. And there is no doubt in their minds that the action is needed. □

International science (2): OAS

Science for progress

Mahindra Naraine traces the history of the OAS's involvement in science in Latin America

THE Organisation of American States (OAS) is probably the least well known of the major international organisations. United States hegemony over the nations in the western hemisphere has resulted in scant attention being paid by 'outsiders' to the OAS and its operations. Yet it is interesting that the OAS is the oldest international organisation of its kind in the world. Not only that; for the past ten years it has also had a Scientific and Technological Programme. With a budget in 1976 of about \$10 million (about 20% of the OAS budget), this programme supports such work as the development of solar refrigeration in Mexico, the study of fur seals in Uruguay, computer technology in Brazil and deforestation studies in Haiti. In comparison with this the science and technology efforts of some other organisations seem puny.

Although the OAS has been in existence for some time its main pre-occupation has been the idea of hemispheric peace, naturally under the guiding hand of the United States. The Cuban revolution and the Kennedy Administration together helped to speed up efforts aimed at the economic sector. The much-acclaimed Alliance for Progress, set in motion in August 1961 at Punta del Este (Uruguay), had the intention of eradicating economic under-development in Latin America. By the late 1960s corruption and maladministration minimised the potential benefits of the influx of some \$1,000 million into the region. The programme did not work well, and by 1967 it was obvious that a political boost to the OAS was necessary.

The American Chiefs of State met that year and in the 'Declaration of Punta del Este' stated that their aim was the creation of a Latin American Common Market. This was to begin in 1970 and to be in operation no later than 1985; it was to be a convergence of the then existing Latin American Free Trade Association and of the Central American Common Market system. The declaration also expressed the intention of harnessing science and technology for the service of the Latin American peoples. To effect this it was decided to establish a Regional Scientific and Technological Programme (PRDYCT). It was also stated that because of the magnitude of the

investments required for science and technology, it was necessary to have inter-American cooperation.

In compliance with the Punta del Este Declaration, the Inter-American Cultural Council formally established PRDCYT in February 1968. In this Resolution of Maracay it was stated that PRDCYT

... shall be oriented towards the adoption of measures to promote scientific and technological research, teaching and information, basic and advance training of scientific and technical personnel, and exchange of information. It shall intensively promote the transfer to and adaptation by the Latin American countries of knowledge and technology originating in other parts of the world.

PRDCYT was not the first programme of a technical nature to exist in the OAS. As far back as 1950 a programme of Technical Cooperation was created to deal with requests for technical assistance. With the advent of the Alliance for Progress there was a rapid expansion of technical assistance projects. The OAS then established a special training programme in which, for the first time in OAS history, countries outside the hemisphere could participate. Several European countries participated, but the most significant contribution in this category has come from Israel. In 1975, for instance, Israel provided 70% of the experts for technical co-operation from non-member countries. Mechanisms for the operation of

PRDCYT were somewhat predetermined by the imperatives of integration and infrastructure. PRDCYT set out to create centres of excellence for the training of personnel from all the member countries. Three mechanisms were involved: multinational projects, supporting actions and basic studies.

Multinational projects are activities that are carried out in one or more centres in the region with the cooperation of several or all member states. Activities could be of a regional, sub-regional or national character. Regional activities are carried out in selected centres and in general consist of special courses, seminars and other types of technical meetings. Activities within the national sphere are meant for institutional development and to improve research and education. The OAS identified two kinds of centres: a responsible centre operated as headquarters for regional activities and provided assistance at a national level to less well endowed centres; centres that received these benefits were called participating centres.

For the less well endowed centres 'supporting actions' were expected to strengthen the scientific and technological infrastructure by building up equipment and other resources. The third mechanism, basic studies, was meant to obtain information on the region's scientific and technological capability and development.

Main emphasis

In the early years of PRDCYT the main emphasis was on multinational projects. These were allocated about 60% of the budget, in keeping with the big push for Latin American integration. There were multinational projects on mathematics, physics,

A union of American Republics has existed since 1890 but it was only in 1948 that a Charter was drawn up and the Organisation of American States created. The OAS consists of 25 member countries spread over two continents and six islands in the Caribbean. Cuba still remains a member but in 1962 was excluded from participation in OAS affairs.

Early attempts were made by Simon Bolivar to develop the ideal of regional solidarity, but these failed. It was not until the Monroe Doctrine and the idea of the Western Hemisphere as the region of United States influence that hemispheric unity, even if somewhat unbalanced, was feasible. The OAS has therefore suffered the criticism that most of its actions have been for the benefit of the dominant partner—the US.

OAS headquarters are in Washington; 18 of the member countries are Spanish-speaking. Other related OAS organisations include:

The Pan American Health Organisation (PAHO). Based in Washington, this was founded in 1902 and promotes and co-

ordinates hemispheric efforts to combat disease, lengthen life and promote health in general. PAHO also serves as a regional agency of WHO and maintains close relations with the national health services in member countries. In 1974–75 its budget was \$34 million.

The Inter-American Institute of Agricultural Sciences (IICA). Founded in 1942 with headquarters in Costa Rica, it is the specialised unit of the OAS for agriculture and does teaching, research, technical assistance and conservation. Its activities are spread over the four geographic zones of the region. IICA manages the Simon Bolivar fund set up for rural development.

The Inter-American Nuclear Energy Commission (IANEC). This is a technical commission set up in 1959 to serve as a centre of information and consultation for the member states and to offer them the necessary cooperation and assistance for the peaceful uses of nuclear energy. The commission is financed mainly by funds from the regular budget of the OAS.

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chemistry, genetics, biochemistry and so on. Not all the countries benefited equally from the project, however. The more developed countries had had a greater exposure to science and made more use of the new opportunities.

The main criticism, however, came from Latin American academics, mainly social scientists. Continued pressure resulted in the OAS sponsored 'Conference on the Application of Science and Technology to Latin American Development' (CACTAL). This conference was held in 1972 in Brazilia and its recommendations, called the Consensus of Brazilia, had a major impact.

Essentially CACTAL posited that development of a scientific and technological infrastructure that was not very closely linked to the productive sector was fruitless. What was important was that national science policies be closely coordinated with economic and social policies. There must be removal of technological dependence on the developed nations, indigenous technologies had to be developed, teaching and research should be geared to the labour market and the productive sector, scientific and technological development should give preference to the rural and urban marginal sectors and national science policies should look at such matters as patents, trademark legislation and technology transfer agreements.

The OAS noted the resolutions and attempted to modify its programme. The OAS 'Mar del Plata Resolution' acknowledged that the structure of PRDCYT was too rigid to meet the demands of the member states in a timely manner. It recommended that more attention be paid to economic and social development; for this there would be a revision of the fields of action and types of activities. It was agreed that there was an urgent need for scientific and technological development in the developing countries; for this a special Mar del Plata fund was created giving preference to them.

Funding

The nature of the funding of OAS activities is somewhat complex. There is an OAS general fund to which all members contribute according to quotas established by the OAS secretariat. The total budget for 1961 was \$8.3 million. In 1968 it was \$17.2 million and in 1975 \$56.5 million.

PRDCYT is administered by the Permanent Executive Committee of the Inter-American Council for Education, Science and Culture (CEPCIECC). CEPCIECC works out a budget for its fiscal period in line with previous years and proposed projects. Members are then asked to

pledge amounts to this CEPCIECC fund in a similar ratio to the OAS general fund. Contributions are voluntary. Typical ratios are 0.19% for the poorest countries, 7.4% for countries like Brazil and Argentina, and 66% for the United States.

The CEPCIECC budget is for science, education and culture. Until 1974 PRDCYT was allocated 60% of the budget but this figure has since been reduced to 50%. It has been said that the change is a consequence of most of the representatives on CEPCIECC being Ministers of Education. In its first year of operation PRDCYT was allocated \$2.4 million excluding administration costs. In the first three years the programme disbursed some \$18 million. For the next five years over \$50 million was spent.

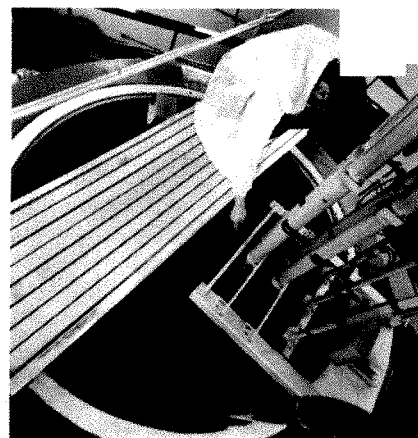
The funding has been further complicated by the creation of the Mar del Plata fund in 1974. Under this each country has a multiplier factor; the poorer the country, the higher the multiplier. Countries whose quota to the general OAS fund is less than 0.75% have a multiplier of about 7; for a country like Mexico it is 1.9. A country then allocates money to this special fund providing it does not exceed the sum it contributes to the regular fund. This contribution is then increased by the multiplier factor; the matching funds are made up mainly by the United States. For the 1974-76 period the Mar del Plata account had some \$5.8 million.

At the present time PRDCYT acts in areas of basic and applied sciences (supporting the funding of research projects and training of personnel), technological research and the transfer of technology. It is also involved in scientific and technological policy and planning—helping in the formulation of national science policies.

Applications for support are sent through the national science agencies to the OAS. The OAS has a panel of experts which provides technical guidance on the application. Projects can request training of personnel, recruitment of visiting professors, equipment, books and so on, but do not cover administration expenses or other requirements. In compliance with political attempts at integration, however, preference is given to projects that are multinational, so that proposals always include provision for contact or assistance with other regional institutions or personnel.

Main problem

Ironically, PRDCYT's main problem has been its association with the OAS. The programme was developed through political actions and it has never lost that association in the eyes of its critics. The United States' dominance



Bolivian reactor project

of the OAS and its manipulation of the region to secure hemispheric solidarity has raised many doubts about the worth of any OAS programme.

Undoubtedly, the benefits have not been as large as they might have been. The more developed countries have benefited most from the programme, but this should be expected since they have a better science and technology infrastructure. The Mar del Plata account has helped to redress the balance, but the main issue here is that it limits the amount of money countries can obtain. The newly created Simon Bolivar fund, financed mainly by Venezuelan petrodollars, will concentrate its efforts on rural development—PRDCYT's main problem is that it has not been closely linked to direct economic development. Criticisms of it on technology transfer conditions, patent legislation and types of development, however, are spurious, since these are issues that have to be settled internally by national policies. Awareness of these problems on the part of some national agencies has actually stemmed partly from PRDCYT-organised courses, but these agencies find it difficult to convince their superiors of the need for integrated science policies.

PRDCYT has been important in the development of the scientific and technological infrastructure of the Latin American nations. Through its efforts many centres of excellence now exist that are the prerequisites for the generation of indigenous technology. Many institutions rely on the programme for financial support for research, since they receive very little money directly from their own governments. According to Article 45 of the OAS Charter, governments should encourage science that is oriented toward the overall improvement of the individual—that is a foundation for democracy, social justice and progress. In too many Latin American countries money is used for the very opposite purposes. □

Danger: men at work

Judy Redfearn looks at the problems facing those concerned with major hazards in Britain

ALMOST three years ago an explosion at a Nypco factory in Flixborough killed 28 people and injured more than 100. It shocked the British public into awareness of the possibility of other major industrial accidents. The Health and Safety Commission's Advisory Committee on Major Hazards was set up in the wake of Flixborough to lay down recommendations for the control and supervision of major hazards. Nine months ago it published its first report, which helped those concerned with industrial safety to think seriously about its recommendations and how to implement them. Some of the problems that report brought to light have since been discussed, notably at a meeting organised by the Institution of Mechanical Engineers at the beginning of May, when representatives from industry, local authorities, the Health and Safety Executive (HSE) and the committee itself got together in London.

The report's main recommendation poses a major problem. The recommendation was that a 'notification' scheme should be set up to help the HSE compile a list of major hazards: all installations would be subject to review, even though most would demand no more regulation than already exists under the 1974 Health and Safety at Work Act; but potentially hazardous installations would be required to submit a detailed survey of their activities to the HSE. At the moment, this is voluntary, but the committee would like to see it enforced by legislation.

The problem with the scheme is the burden it will impose on the HSE. Not only will the Executive receive a large number of surveys from all kinds of installations; it will also need to examine in great depth those which might indicate a major hazard. To relieve some of this burden, the committee has suggested that individual companies should be responsible for indicating their own problems and outlining the solutions. But this still means the HSE will need more specialised staff. The committee has suggested that outside expertise should be made available at any time.

Another difficulty is that it is not yet clear how the top league of hazardous installations would be controlled: whether by regulation or licensing. The committee feels that it can only wait until both local authorities and the HSE have acquired, under the notification scheme, fairly comprehensive in-

formation on the types of activity they will be controlling and on the problems they will be facing before it can recommend which way to go. One of the dangers it wishes to avoid is transferring liability for major accidents from industry to the regulating body. Whatever machinery is set up, some industrialists feel that a major stumbling block will come in trying to ensure that their special case is understood by whoever is to judge it. And in the end what does or does not constitute a major hazard will be someone's subjective judgment.

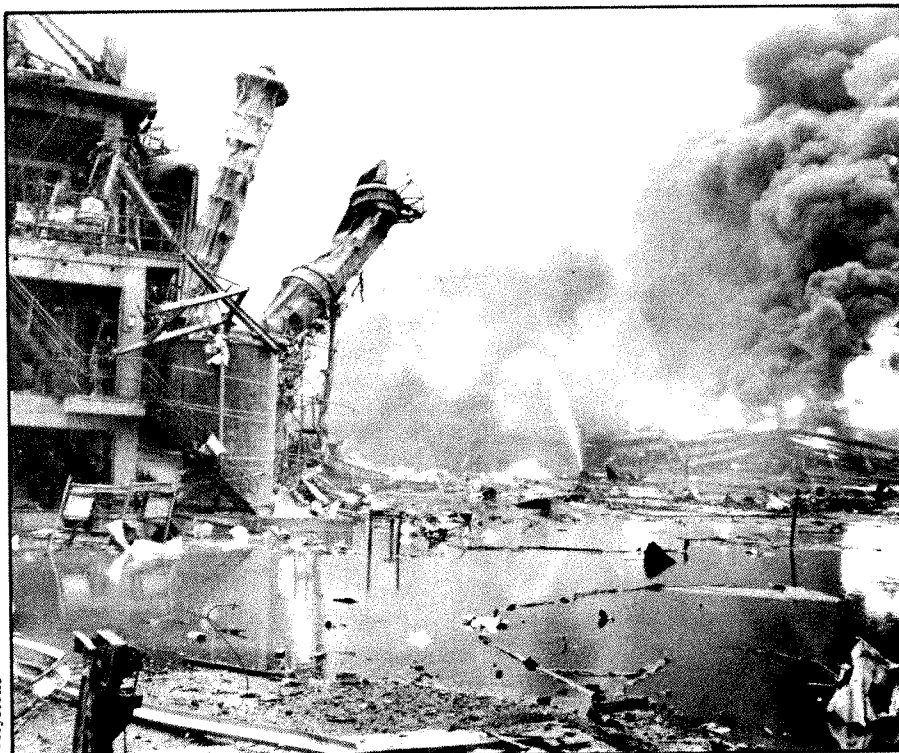
Nor is this all. Accidents happen—either through the fault of people operating machinery or through mechanical breakdown—but the extent of the damage they cause depends on environmental factors. The report recommended that new plants should be planned to take account of the likely consequences of a major accident: buildings should be capable of withstanding shock from explosions and of containing toxic chemical leaks and should be situated away from densely populated areas. Machinery should be designed to the highest specifications and staff should be continually retrained in operating procedures.

The trouble is, human error can never be eliminated, whether in the

design or in the operation of an industrial plant. The recent meeting produced a general call for more research into the relative safety of automatic control systems and human control: it was asked, for example, whether it was safer to leave everything to machines and to have no one about when an accident does happen, or to have somebody there to warn others when something goes wrong. The committee is also concerned about the lack of knowledge on the massive release of toxic and flammable gases that are heavier or lighter than air.

Industry expects other problems once a plant has been labelled a 'notifiable installation'. For example, people have to be convinced that it is safe to work there. The feeling is that if a reputation has been established for giving reliable information on all aspects of its work, employees are likely to trust what is said about safety. The difficulty is that too often in the past industries have been found to have wrong statistics even on fundamental points. Public confidence will only be inspired if industries are seen to be reliable. A possible way of educating the public might be through the unions, but they have yet to be approached.

The committee's work is still in its first stages. It plans to hear more evidence and produce other reports as and when it sees fit. It still has many points to clarify before it can recommend further action and through the HSE it is liaising with the Dutch—the only other nation looking into major hazards. In the meantime it is simply waiting to see what turns up. □



Devastation at Flixborough

ITALY

Distant drums

The pollution threat posed by the cargo of a submerged ship in the Adriatic could now be receding. Alastair Hay, recently in Italy, reports

FOLLOWING a collision with a Panamanian ship in July 1974, the Yugoslav vessel *Cavtat* sank three and a half miles off the coast of Otranto, in southern Italy. The *Cavtat* was carrying as part of its cargo 270 tons of the petrol 'anti-knock' additives lead tetraethyl and lead tetramethyl. These were stored in drums and located in the hold and on the deck. Three years later, most of the drums are still there—under three hundred feet of water at the entrance to the Adriatic.

The pollution problems of the Mediterranean are legion. Those of the Adriatic are even more numerous. Industrial effluent discharged directly into the Adriatic without prior processing has led to an increasing accumulation of heavy metals, particularly of lead, zinc, cadmium and mercury. The Italian government has taken steps to control the discharge of untreated effluent. However, equal concern was not at first in evidence for the pollution threat posed by the leakage from the drums in the *Cavtat's* cargo. Indeed, it took the government more than two and a half years to act: on 3 February this year, following a midnight cabinet meeting, the government finally announced that £6.7 million was being set aside over a two-year period for salvage operations to raise the drums.

Several factors forced the government to take this decision: a growing realisation that the cargo, if left undisturbed beyond the spring of this year, will be impossible to salvage; publicity about the pollution hazard which is having a serious effect on the local tourist and fishing industries; and a vocal Otranto magistrate, Alberto Maritati. Shortly after the accident,

Maritati began an investigation and sought scientific advice on the hazards posed by the lead compounds. Most scientists agreed that, if the chemicals were to escape from their drums, a major ecological disaster could well ensue. But opinion differed as to the advisability of raising the drums to the surface. Some considered that the drums were better left on the sea bed and perhaps carpeted with a layer of concrete. The British firm of Octel, who manufactured and packed the lead products, considered the *Cavtat* problem to be a storm in a teacup, the company's technical manager reportedly having said that "... the best thing to do is to leave the drums down here. If it was all released at once, it wouldn't cause much of a problem in my opinion. I think it is an exercise in sheer futility".

This, however, has not been the majority view of the problem. Reports from marine scientists and toxicologists confirmed Maritati in his opinion that the lead compounds were hazardous and could enter the marine food chain. He attempted to obtain government support for his decision to raise the *Cavtat's* lethal cargo. Despite dozens of journeys to Rome and many formal written requests for assistance he received little support for his project. Maritati had powers invested in him as a magistrate to initiate the salvage programme, but he had no funds to pay for it. This was the government assistance he sought. Finally, on 26 January, and still without the promise of government aid, Maritati issued an order requiring the Saipem company—experts in underwater engineering, and a subsidiary of the Italian state oil company, ENI—to begin salvage operations. The magistrate was convinced of the need for urgency after the inspection of a drum raised in December by a team of divers had revealed corrosion of the containers and confirmed their porosity; it seemed essential that all the drums be brought to the surface before further corrosion made the operation too hazardous.

Interviewed recently, Maritati told *Nature* that he required no additional assistance from the central government. A Communist Party member dealing with Christian Democrats, he said his efforts to enlist government support had resulted in an assurance that it would pay for the entire salvage operation. He was told that there is now an arrangement between the Department of the Merchant Navy and

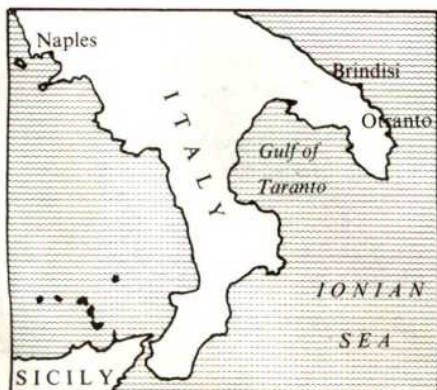


Poisoned fish caught in the Gulf of Taranto

Saipem "that all the drums will be raised". Saipem is working with the manufacturers Octel, who will handle the drums when they are brought to the surface.

The most difficult part of the salvage process will be to secure the drums in the ship's hold. However, Saipem are convinced that the problem is not insuperable and have assured Maritati of this fact. Maritati says that it is "a problem which requires urgent attention. I visit the site every day to ensure that the operation is proceeding to my satisfaction. There should be no interruptions in the salvage operation. This being so, all the drums will be raised by June. And so tourists will have nothing to fear".

Once the cargo is neutralised the *Cavtat* problem will still feature prominently in the courts. The captain faces criminal prosecution on two counts: that of polluting territorial waters, and of criminal negligence—he is alleged to have refused the help of Italian tugs which could have towed his ship to safety. Maritati will be involved in this legal process, but for the moment he has won his case for government assistance. The government, too, is anxious to bring the matter to a speedy conclusion. Officials in the Ministry of Foreign Affairs were insistent that central government hesitation on the issue was not due to any deliberate obstruction, but to the fact that so many departments were involved in the affair; consultation with and between all the departments was essential before any action could be taken. That action is finally in view. Everyone hopes that the outcome will be a potentially cleaner Adriatic. □



USA

●A key advisory committee last week decided to recommend that guidelines which now govern recombinant DNA experiments in the United States should be amended to facilitate experiments with human DNA. Though the committee's recommendation, which will eventually be made to the Director of the National Institutes of Health (NIH), may not be accepted, it is sure to generate considerable debate.

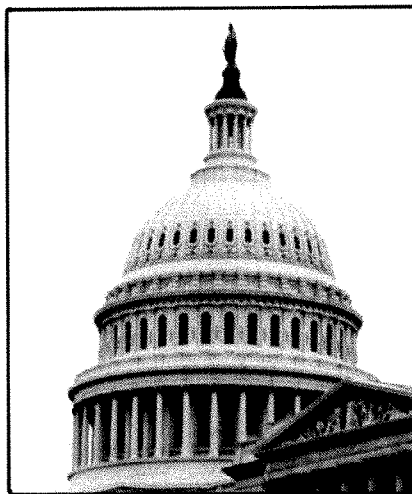
The committee, the Recombinant DNA Advisory Committee, carries considerable weight because it was largely responsible for drafting the present NIH guidelines. The guidelines require that recombinant DNA experiments involving human genes must be performed in the highest level of physical containment (P4), and in addition, crippled strains of micro-organisms must be used (a so-called EK2 system). Those requirements effectively bar work with human genes for the time being because no P4 laboratories have yet been certified, and few universities are likely to build them. The advisory committee will recommend, however, that the containment level should be reduced to P3 and EK2, a level which would allow such work to go ahead in many places.

The committee decided to recommend the change for a variety of reasons, and its vote was 11 to 1, with one abstention. A number of committee members told *Nature* last week that they agreed to recommend the change because they feel that evidence, accumulated since the original guidelines were drafted, indicates that potential hazards associated with many types of recombinant DNA research are extremely remote, and work with human DNA is likely to yield important results. In particular, Roy Curtiss of the University of Alabama, who proposed the change, argued that so far nobody has found faithful expression of vertebrate DNA in *E. coli*, that if cloned DNA were to get into the intestine it would rapidly be degraded by enzymes in the gut, and that several experiments suggest that *E. coli* containing 'foreign' genes is at a selective disadvantage.

The move is sure to be criticised on scientific as well as political grounds, however. Experiments involving human DNA were assigned to the highest level of physical containment largely because of the possibility that human cells may harbour cancer viruses whose genes might be picked up and cloned along with the human DNA, but some of the advisory committee members noted that so far an

intensive search for human cancer viruses has failed to find any. That potential hazard cannot be entirely ruled out however, and the recommended relaxation of the guidelines is therefore likely to be controversial.

The committee will meet again in June to discuss further recommendations to amend the guidelines, and its proposals will eventually be sent to



the NIH Director Donald Fredrickson, who will almost certainly subject them to public discussion before acting upon them. In the meantime, Congress is busy working on legislation to regulate recombinant DNA experiments, and it is likely to require that new regulations be drafted. It could take as long as 18 months for new regulations to be drafted and implemented, however, so the NIH guidelines are likely to be important for some time yet.

●Meanwhile, in a move which is sending a few tremors through the biomedical research community, the environmentalist organisation Friends of the Earth, has gone to court to seek an order halting federal funding of recombinant DNA research in the United States. The organisation has charged that NIH violated the law by failing to publish an environmental impact statement before it issued its recombinant DNA guidelines last year.

The law in question, the National Environmental Policy Act, requires that government actions likely to affect the environment must be preceded by publication of an analysis setting out the potential environmental impact and discussing alternative courses of action. NIH issued its recombinant DNA guidelines in July, and it published a draft environmental impact statement six weeks later. A final version of the statement is now

under review in the Department of Health, Education and Welfare and it has yet to be published.

The chief reason why the process got out of step was that NIH didn't realise until the guidelines were in the final stages of being drafted that an environmental impact statement would be required. A long delay in issuing the guidelines would therefore have resulted if NIH had followed the letter of the law.

When the draft environmental impact statement was published in August, NIH Director Donald Fredrickson admitted that the legal process had been short-circuited, but he argued that the public interest was best served by prompt issuance of the guidelines. Recombinant DNA experiments were then proceeding under general safety rules recommended by a group of geneticists which met at Asilomar, California, early in 1975, and since the NIH guidelines are more strict than the Asilomar rules, Fredrickson argued that "the escape of potentially hazardous organisms was more likely in the absence of NIH action".

Friends of the Earth is claiming that because NIH failed to follow the procedures laid down by the National Environmental Policy Act, the guidelines were issued improperly and should be withdrawn. Federal funding for recombinant DNA experiments should then be halted, the suit demands, pending the resolution of safety issues.

NIH has 60 days to respond to the suit, and lawyers from the Department of Health, Education and Welfare met last week to discuss their response. Even if the courts find that NIH violated the letter of the law, however, they need not necessarily require a halt to the research.

●A bill to provide a major infusion of funds into earthquake prediction and into the design and construction of earthquake resistant buildings, dams and other structures, was approved unanimously by the Senate last week, and it has also been passed by a key committee in the House. The bill, which has been kicking around Capitol Hill for several years, is thus virtually certain to clear Congress in the next few weeks. It would authorise expenditure of \$55 million next fiscal year and a total of \$205 million over the next three years for earthquake research, and it directs the President to draft an implementation plan and to recommend ways to help communities prepare for earthquake warnings.

Colin Norman

COMECON

Space relations

The Comecon countries' joint space programme includes a manned mission planned for next year. Vera Rich describes preparations

THIS year, which marks the twentieth anniversary of the first Soviet satellite Sputnik 1, is also the tenth anniversary of the joint Comecon 'Interkosmos' programme. To date, the achievements of Interkosmos have amounted to the launching of 16 orbital probes and four high-altitude rockets. Last year, however, a major new development was announced: the Soviet manned space programme would be extended to include cosmonauts from the other member countries of Comecon.

The first trainees, said to be from Poland, East Germany, and Czechoslovakia, arrived early this year at the 'Star City' training centre near Baikonur, and candidates from other Comecon countries are joining them during the course of the year. As the first manned Interkosmos mission is planned for 1978, the training programme is clearly a considerably abridged version of the normal Soviet five-year course, and it is therefore not surprising that from the start it has been stressed that each joint mission will be captained by a Soviet cosmonaut.

The prospect of a fellow-citizen in orbit has done much to rekindle interest in space research throughout the Comecon bloc. A recent exhibition in Warsaw of Soviet scientific achievements included a special 'Kosmos-77' pavilion which, in the course of five weeks, attracted more than 1.2 million visitors. The Soviet 'Cosmonautics Day' festival—the anniversary of Gagarin's flight—fell during this exhibition and was celebrated with fitting honours, including a visit from Professor Roald Z. Sagadiev, Director of the Institute of Space Research of the Academy of Sciences of the USSR, and cosmonaut-pilot Viktor V. Gorbachko, captain of the recent Soyuz 24 mission.

The participation in the not-too-distant future of a Polish cosmonaut in a joint mission might well have been expected to form a major theme of the speeches on this occasion. However, Professor Stefan Piotrowski, Head of the Committee for the Investigation and Peaceful Uses of Space of the Polish Academy of Science, chose to concentrate on the special 'Kopernik 500' Interkosmos launch of April 1973, "the most important Polish space experiment to date", for which, in

honour of the Copernicus quincentenary, "almost all the apparatus and scientific programme" was produced by a Polish team. From the Soviet side, Professor Sagadiev spoke largely of such future prospects as deep-space probes to the outer regions of the solar system, orbital laboratories, and satellite monitoring of weather, crop prospects and pollution. The proposed launching order of the Comecon cosmonauts remains a closely guarded secret.

This is, of course, in accordance with Soviet practice which normally never permits the disclosure of details of a mission in advance. There is undoubtedly unofficial rivalry between the Comecon countries as to whose cosmonaut will blast off first, and a minor set-back in training or a last-minute indisposition leading to a change in order might lead to undesirable tensions and ill-feeling between the countries concerned.

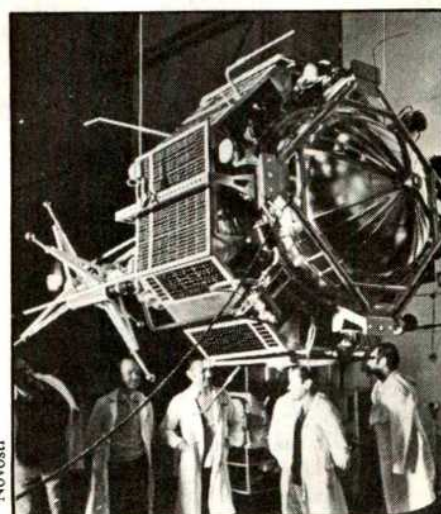
It is noteworthy, however, that the three countries whose representatives arrived first at the training centre are those which have been most closely concerned with the unmanned Interkosmos probes, providing instrumentation and experiments for the satellites. East Germany, in fact, not only supplies electronics and telemetry systems for the Interkosmos satellites, but also in September 1976 provided a special 'multispectrum' camera for the Soviet Soyuz 22 mission.

The final goal of the Comecon bloc is total economic integration, and although the current five-year plans are temporarily playing down this aim, concentrating rather on a short-term (15–20 years) drive towards integration in certain significant areas, one of these chosen fields is instrumentation. The selection of East German, Czechoslovak or Polish equipment for Interkosmos satellites is an outcome of this policy. Since, under the complicated financial arrangements involved, it is the supplying countries who pay for the R&D, the planners might feel that priority given to the cosmonauts from these countries would be a graceful compliment to their considerable effort and outlay. It would also be a way of stilling the rumours of discontent that the other Comecon members are paying the lion's share of grandiose projects initiated by the Soviet Union and for which the Soviet Union gains the acclaim.

It should be remembered, however, that in spite of special satellite tracking ships, the Soviet Union relies heavily on ground bases in the Come-

con countries. Because of their geographical location the two least-developed members—Cuba and Mongolia—are particularly important in this respect; indeed, Cuba joined the Comecon 'Intersputnik' communications systems when the latter was first set up in November 1971, eight months before becoming a member of Comecon itself. Under the Intersputnik agreement ground stations are owned and operated by the countries in which they are sited. Although as developing countries Mongolia and Cuba are said to receive the necessary Intersputnik facilities from the Soviet Union free of charge (unlike the European members of Comecon which must bear the cost themselves), the value of the Havana and Ulan-Bator tracking bases to the Soviet space programme is not expressible in roubles, and the inclusion of Cuban and Mongolian cosmonauts in the training programme reflects a genuine contribution made by these countries and is not simply a tribute to the principle of the 'friendship of nations'.

Although the joint teams of the Interkosmos manned missions will presumably carry out the now routine geophysical and astrophysical observations, medical checks and, possibly, experiments on the effect of weightlessness on physical and chemical processes such as crystal growth, it seems unlikely that the non-Soviet cosmonauts will contribute any special knowledge or expertise which their Soviet counterparts could not have provided. Rather is it a grand exercise in public relations, to demonstrate the fraternal cooperation of the Socialist countries—and to provide for those countries a greater involvement in the Interkosmos programme than equipment on board a Soviet-built satellite or a tracking station on their soil. □



Inspecting Interkosmos 10

UK response

foreign uranium supplies, uranium enrichment as a source of weapons, development of international reprocessing facilities and storage of unprocessed fuel, he was apparently serving notice that the political battle to win a consensus could be drawn out.

UKAEA plans

Apparently in response to fears expressed by critics of nuclear power over the threat of plutonium diversion, the UK Atomic Energy Authority (UKAEA) is planning to demonstrate two methods of combating the problem. Speaking last week, the UKAEA chairman, Sir John Hill, said that the UKAEA was proposing to build a

finement. *Aviation Week* cites the revelations by the Soviet physicist Leonid Rudakov last summer during a tour of US fusion laboratories. Rudakov, "in response to a taunt by a western scientist", revealed that

[illegible]

The test site that is crucial to General Keegan's thesis is at Semipalatinsk near the Tibetan border. *Aviation Week* describes in detail a large building containing two steel spheres and states that since November 1975 seven tests "that may be related to development of a charged-particle beam device" have been carried out. The only evidence for the tests cited, however, is the hydrogen with traces of tritium in the upper atmosphere—which could mean almost anything.

According to *Aviation Week* the Russians have made advances in explosive or pulsed power generation, flux compression, pressurised and cryogenically cooled power cabling, very large capacitor construction, energy switching, electron injection and collective acceleration. Together these advances would give them the ability to produce a proton beam intense enough to be used as a weapon. It is claimed that for some of those technologies the Russians are farther advanced than the Americans; in others General Keegan says the Russians have access to US advances which they were previously believed not to know about.

"the USSR can convert electron beam energy to compress fusible material to release maximum fusion energy".

But this is far from the description of the event given by other high energy physicists, who say that Rudakov merely gave technical details (which happened to be classified in the US at that time—hence the fuss) of a Russian experiment that had been widely reported in the West several months earlier. True, in that experiment the Russians had for the first time produced some neutrons in an attempt at electron beam fusion, an achievement that the Americans have not yet matched. It is doubtful that Rudakov blurted out his secrets by accident; he stood to gain much more in international prestige than he lost by indiscretion. For the same reason high energy physicists believe that, as the Russians

Another aspect of the technology *Aviation Week* touches on is accelerator design. For a land-based beam there is the perhaps insurmountable problem of propagation through the atmosphere without losing all its energy. A satellite-based beam is therefore an attractive proposition but would limit the size of the extremely powerful accelerator that would have to be carried on board. Theoretically there are several ways of decreasing the size of an accelerator; they include the idea (originating in the Soviet Union) of a collective accelerator in which the protons that will form the beam are initially surrounded by electrons which drag them along and are more easily accelerated; the electrons are then blown off to leave the beam.

This concept has received considerable attention both in the Soviet Union and in the United States but has now been largely discarded. The 'smoke ring' collective accelerator experiments at Berkeley, for example, have stopped because of enormous problems in creating a stable, sufficiently intense electron beam. Interestingly, *Aviation Week* fails to discuss any evidence of Soviet progress in that area. It seems therefore that at least one crucial component of a satellite-based beam weapon is not yet technologically feasible.

Even if General Keegan's assessment of Soviet technology in most areas is substantially right, there remains the claim of whether the ultimate aim is a beam weapon. The interesting thing about *Aviation Week's* list of technologies is that they are all equally crucial for the development of fusion by an electron or laser beam. The location that General Keegan interprets as a beam weapon test site is equally likely to be used for fusion experiments. And being first with fusion would confer a good deal more power than ownership of a decidedly cumbersome and delicate weapon.

fuel fabrication plant at Dounreay, now the site of Britain's 250 MW prototype fast reactor and of a fuel reprocessing plant. This would eliminate the need to transport plutonium once the initial charge is delivered.

The UKAEA also hopes to demonstrate the concept of a single reprocessing plant servicing a "substantial number" of reactors. The idea of irradiating reprocessed and refrabricated fuel before it is dispatched back to a reactor is apparently under consideration, but there may, according to Sir John, "be easier, more convenient ways of achieving the same objective developed in the interim".

Convention signed

Representatives of over thirty countries, including the United States and the Soviet Union, gathered in Geneva last week to sign an international convention known as Enmod which prohibits the hostile use of environmental modification techniques. The convention, which took three years to negotiate, allows research and develop-

ment on the manipulation of the environment for peaceful purposes.

ACOPS report

A 20% increase (500 to 595) in the number of pollution incidents around UK coasts between 1975 and 1976 is reported in the annual report of the UK Advisory Committee on Oil Pollution of the Sea. The report, out last weekend, expresses disappointment at the lack of progress at the UN Law of the Sea Conference, another session of which opened this week in New York.

More teaching companies

To improve the interaction between industry and universities, the UK Science Research Council (SRC) and the Department of Industry (DoI) have agreed to expand the Teaching Company Scheme over the next five years. They will share the cost equally and have made provision for a maximum of £2 million to be spent in any one year.

First proposed in 1975, the scheme was designed to introduce able grad-

uates to industry under the joint super-industrial workers, in the hope that vision of university academics and they would remain and follow careers in management. Initially, the scheme was taken up by five companies in cooperation with nearby universities and although these programmes have suffered problems it has been decided to go ahead with new ones. Two more schemes were approved last week: Lesney Products in cooperation with North East London Polytechnic, and Anderson Strathclyde Ltd in cooperation with the University of Strathclyde. The target is now twenty teaching companies by 1982.

Asbestos meetings

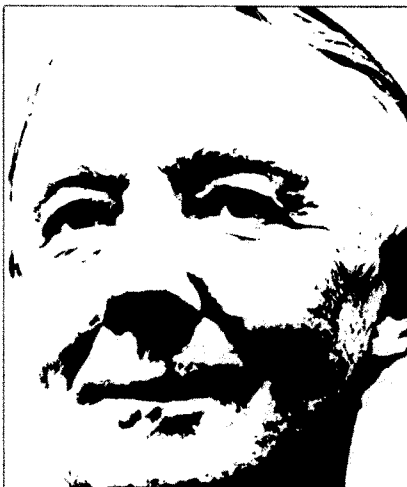
The UK Advisory Committee on Asbestos, set up in March last year by the Health and Safety Commission to review the risks to health from exposure to asbestos, has invited individuals and organisations who have submitted written evidence to attend three public meetings the committee is holding in London next month.

THE world's oil deposits are finite, and are likely to be exhausted within the next hundred years. Pessimists suggest that they may only last for twenty-five years, optimists believe there are supplies for two or three times as long. In the past oil has caused a great deal of environmental damage, including air pollution from refineries and spills from wrecked tankers such as the *Torrey Canyon*. The recent blowout at the Ekofisk rig in the North Sea is a reminder of continuing dangers which are unavoidable. It is important to ensure that this short-lived, though important, industry does not leave behind a permanently damaged world.

Although the oil industry exists to make a profit, the major companies have for some years taken a responsible attitude to the environment. A recent manifestation of this is the establishment of the Conoco Lecture by the Continental Oil Company, one of the major international entrepreneurs in this field. The first lecture was by Dr Peter Chapman, of the Energy Research Group of the Open University, on "The responsibility of industry towards the environment". He demonstrated how important it was to consider all stages in, for instance, energy generation, when trying to reduce pollution. A comparatively clean power station might depend on fuels and machinery which had already produced massive pollution. He stressed the point that

'market forces' themselves could not be relied on to protect the environment, and that industry must be prepared to accept restraints which may

Duty on oil



KENNETH MELLANBY

reduce its profitability.

Pollution control is very complicated, and few generalisations can be widely applied. Legislation may or may not be effective. The first British Alkali Act of 1867 was strongly resisted by industry, but was effective in reducing the serious damage done by hydrochloric acid from alkali works. The Clean Air Act of 1965 is generally given the credit for clean-

ing the air of London and other cities; this myth was again propounded by Dr Chapman. Actually the amount of smoke and dirt in the air in Britain had been falling rapidly for ten years before 1956, and the rate of decrease was actually slower, not faster, after the Act was passed. It would be an abuse of the statistics to suggest that this law actually prevented air pollution from being controlled, but the main causes of the improvement were economic. By 1956 much of industry had already changed from burning raw coal to using oil, gas or electricity, all cleaner (and, at the time, cheaper) fuels. The change in domestic heating had been slower, but in more prosperous areas clean methods of central heating were widely used when the Act came into force.

Since 1956 it has been possible for local authorities to declare 'smokeless zones' where the domestic use of raw coal is forbidden. This has undoubtedly made the final clean-up of such areas possible, but smoke continues to pour out of the chimneys of many houses in our poorer areas, or in those where our miners receive—and burn—over ten tons each of concessionary coal a year. The law has been much less effective than have economic forces in cleaning Britain's air. It will be interesting to see the effects on the environment of the proposed expansion of our coal industry.

correspondence

Separating swimmers

SIR,—Within the past few years the electronic timer, capable of responding to time differences of .001s, has replaced the stop-watch, capable of responding to time differences of perhaps .05s. In world class swimming events are now decided and records are established by amounts down to .01s. Detailed regulations for the use of such devices are given in the handbook of the international swimming federation FINA. A recent modification to these regulations, passed at the Montreal Olympic Congress in 1976, prohibits the separation, even for placing, of swimmers by times less than .01s. Clearly, the significance of such accurate timing needs examination.

According to FINA Regulation, a pool for an Olympic or for a World Games must have a length of 50.00 m with a "tolerance of ± 0.03 m". In other words, the maximum permissible error in length, from one pool to another, is $\pm 0.06\%$. This error is recurrent and will remain the same, no matter how many lengths of the pool are swum. If timing is conducted to one-hundredth of a second electronically, that is to ± 0.01 s, the maximum possible timing error in the fastest event, the Men's 100 m Freestyle swum in a typical time of 50s, is 0.02% . The timing error of 0.01s is not recurrent, and so the percentage timing error decreases for longer distance races, taking longer times.

It is well known that the overall accuracy of any measurement is set by the least accurate measurement. For the example above, the Men's 100 m Freestyle, the maximum possible error, 0.06% , is three times the maximum possible timing error of 0.02% , the ratio becoming more unfavourable for longer distances.

It is therefore not valid to compare records made in different pools, unless the times can be corrected to the exact length of the pool, or unless the time difference is, in the case of the Men's 100 m Freestyle, at least 0.03s and correspondingly greater for longer distances up to a maximum of about 0.5s for the Men's 1,500 m swum in a typical time of about 15 minutes. Current listings of world records do not appear to make this distinction, and there is no indication that the records have been corrected to the appropriate pool length. Although in practice many records are established by better

margins, in some cases, especially over shorter distances, 0.01s separation has been the deciding factor.

The figures quoted are typical and not, of course, exact. This does not alter the argument or the general conclusion, however. As surveyor's measurements are usually more accurate than the FINA tolerance of $\pm 0.06\%$, it might be better to specify all records after correction to the 'exact' (that is, the surveyor's) pool length, rather than to attempt to define separation times for each event and every pair of pools. The correction could be programmed into the electronic timer itself. In fact, it is the velocity of the swimmer that is really of interest. Time is used as a convenient indicator, but the assumption is implicit that all course lengths are all known exactly to be equal. Similar arguments may apply in other sports.

Other points might also be mentioned:

- If all the lanes in a given pool are known to be of equal length it may be valid to *separate* swimmers to 0.01s accuracy.

- The well-known problem of the velocity of the sound of the starter's gun is accounted for by separate horns, one at each starting block, each linked to the timer and to the starter's gun. If this is not done, the swimmer in the farthest lane from the gun will start with a disadvantage of some 0.03 to 0.06s, depending on the pool width and the position of the starter along the side of the pool.

- Expansion of the pool with changing water temperature has sometimes been cited as another possible source of error. Water temperature must be maintained at a minimum of 77°F and is unlikely to exceed this by more than a few degrees. For typical materials and temperature ranges the effect of expansion is about an order of magnitude smaller than the pool length tolerance and is not, therefore, significant.

Yours faithfully,

C. H. BARROW

University of the West Indies,
Kingston, Jamaica

Carcinogens and cancerogens

SIR,—The amusing exchange between Lijinsky and Jukes (7 April, page 494) appeared even more droll because they both used the term carcinogen in such a way as to leave question to their

meaning. 'Carcinogen' should refer to the production of carcinomas, that is, tumors of epithelium. Agents that produce sarcomas, leukemias or other neoplasia as well as carcinomas should properly be called cancerogens. I doubt that either correspondent intended to limit their meaning to substances producing only carcinomas—they have followed a widespread but incorrect usage.

Yours faithfully,

CECIL H. FOX

Solna, Sweden

Inexhaustible energy

SIR,—J. Tuzo Wilson (20 January, page 196) has argued that economics must change because the era of cheap energy is past. Many scientists have claimed that the watchword of the next era will be inexhaustible energy. R. W. Cahn (10 March, page 106) has given reasons why fusion power does not really meet this description.

A current campaign, in the United States at least, is to build solar energy collectors in space, and beam energy to the Earth's surface. It is said these would last as long as the Sun does. I feel there must be some flaw in this "inexhaustible energy source" too, and wish someone would tell me what it is.

Yours faithfully,

D. W. ALLAN

Toronto, Canada

Power station in the sea

SIR,—In a short note (31 March, page 402) you say that Loviisa—the location of Finland's first nuclear power station—is south of Helsinki. Have you looked at the map to see where, if this were true, it would be?

Yours faithfully,

STIG-OLOF LONDEN

Otaniemi, Finland

Molecular biology lab

SIR,—There is no truth in your report (28 April, page 768) that the Governing Board of this Laboratory will be disbanded when Perutz retires in September 1979.

Yours faithfully,

M. F. PERUTZ

SYDNEY BRENNER

MRC Laboratory of Molecular
Biology,
Cambridge, UK

news and views

Photosynthesis and photorespiration

from A. L. Moore

DURING photosynthesis, many plants release previously-fixed CO_2 in a light-dependent oxidation process known as photorespiration. The process occurs chiefly in higher plants which fix CO_2 exclusively by way of the C_3 pathway of photosynthesis (the Calvin Cycle). The role of photorespiration is obscure and it is frequently regarded simply as a wasteful process; it has been proposed that net CO_2 fixation by photosynthesis could be increased by up to 50% by preventing photorespiration and the associated oxygen inhibition of photosynthesis. Since all major crop species except maize, sorghum and sugar cane actively photorespire, a major opportunity to increase crop productivity may lie in the control of photorespiration (see Zelitch *Nature* 256, 90; 1975; Chollet & Ogren *Bot. Rev.* 41, 137; 1975), and recent work by Bolhar-Nordenkamp (*Biochem. Physiol. Pflanzen* 169, 121; 1976) aimed at disentangling the complex effects of various environ-

mental factors on the rate of photorespiration and photosynthesis may be an important step towards this goal.

Factors affecting photorespiration include light, high levels of O_2 or low levels of CO_2 in the atmosphere and high temperature. Increases in temperature differentially increase the rate of photorespiration with respect to photosynthesis, whereas photorespiration increases proportionally to photosynthesis as light intensity increases. Bolhar-Nordenkamp has described the interaction of these factors as they affect the conditions necessary for optimal photosynthesis. Figures 1 and 2 show that the greatest light efficiency for optimal CO_2 fixation (CO_2 fixed per given amount of light) occurs at low light intensities, high CO_2 tensions and temperatures near 35°C ; but at atmospheric CO_2 tension (150–400 p.p.m.) the optimum temperature is near 25°C (Fig. 2). However, photorespiration is optimal at high light intensities, high temperatures and low CO_2 tensions. The amount of CO_2 (p.p.m.) in the atmosphere that can be used by the leaf to produce

1 mg CO_2 fixed per g dry weight per h (p.p.m. mg^{-1} in Figs 1 and 2) is optimal between 100 and 650 p.p.m. CO_2 at all light intensities (Fig. 1) and between 150 and 1,000 p.p.m. CO_2 at all temperatures studied (Fig. 2). Similarly CO_2 "fertilisation", which is the proportion of added CO_2 fixed for a given amount of CO_2 in the atmosphere ($\Delta\text{mg}/\Delta\text{p.p.m.}$, Figs 1 and 2), is optimal in the range between 150 and 400 p.p.m. CO_2 at high light intensities (Fig. 1) and high temperatures (Fig. 2). Figure 3 depicts the points of optimal use of light intensity and CO_2 tension for maximal photosynthesis. From Fig. 3 it may be noted that below light intensities of $0.02 \text{ cal cm}^{-2} \text{ min}^{-1}$ and 150 p.p.m. CO_2 concentration, there is no net photosynthesis. This implies that an increase in net photosynthesis can only be achieved by increasing both these factors above the minimal levels. It is also apparent from Fig. 3 that at any given temperature (15°C for example) any variation in either atmospheric CO_2 or light intensity (within the hatched area) will result in the same net photosynthesis. For example at 25°C at light intensities of $0.15 \text{ cal cm}^{-2} \text{ min}^{-1}$, varying the CO_2 concentration between 250 and 1,000 p.p.m. has no effect on net photosynthesis. Similarly at 300 p.p.m. CO_2 and at 25°C , varying the light intensity between 0.05 and $0.16 \text{ cal cm}^{-2} \text{ min}^{-1}$ has no effect on net photosynthesis. But by increasing the light intensity over the range 0.08 to $0.34 \text{ cal cm}^{-2} \text{ min}^{-1}$ and CO_2 concentration over the range 300 to 1,500 p.p.m. at 25°C it is possible to increase net photosynthesis from 25 to 100 mg CO_2 g dry weight per h. Thus only by CO_2 enrichment and high light intensities is it possible to counteract the depression of C_3 net photosynthesis that normally occurs at 35°C .

It is of practical importance that CO_2 enrichment has the added advantage of not only decreasing photorespiration but also increasing nitrogen fixation by symbiotic bacteria in root nodules. It has been suggested that the amount of photosynthate available to the nodule may be the primary factor for limiting nitrogen fixation, and any conditions that increase available photosynthate to the bacteria in the roots would increase nitrogen fixation.

High light intensities in combination

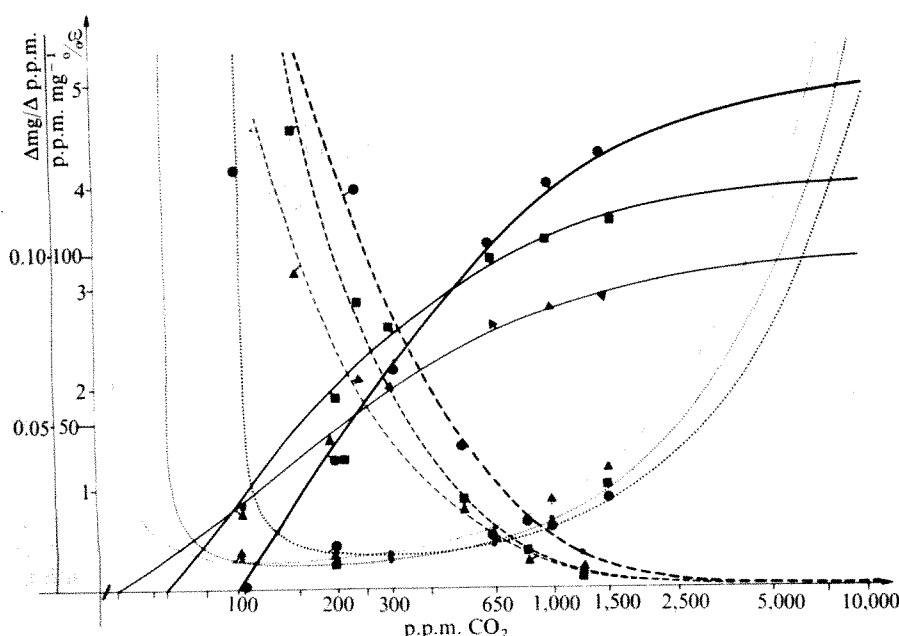


Fig. 1 Effect of CO_2 concentration at four light intensities ($\circ$, 0.556; $\bullet$, 0.265; $\blacksquare$, 0.150; $\blacktriangle$, 0.065 $\text{cal cm}^{-2} \text{ min}^{-1}$) on net photosynthesis at 25°C . —, % light efficiency, ϵ (CO_2 fixed per given amount of light); —, "CO₂ fertilisation" ($\Delta\text{mg}/\Delta\text{p.p.m.}$); , CO_2 in atmosphere used to fix 1 mg CO_2 per g dry weight per h (p.p.m./mg). CO_2 concentration is given in p.p.m. on a log scale.

with atmospheric O_2 pressures (high) and CO_2 tension (low), result in the production of oxygen free radicals during the normal photosynthetic electron transport processes. Bolhar-Nordenkamp suggests that photorespiration occurs as a result of the overproduction of oxygen radicals and the function of photorespiration is thus to prevent any photo-oxidation reactions which could otherwise easily damage the cell. This is thought to be by way of an interaction with glycolic acid metabolism. The mechanism of photorespiration is still controversial, but it is believed that the metabolism of glycolate which may be derived from one or more intermediates of the C_3 pathway, is intimately involved.

Several reactions have been proposed to account for the CO_2 release from glycolate. It is generally accepted that glycolate is oxidised to glyoxylate by glycolate oxidase in the peroxisomes; the glyoxylate is then transaminated to glycine. Glycine is transported out of the peroxisomes into mitochondria where it is converted to serine and CO_2 ; this step is possibly associated with some degree of energy conservation. Recent results in fact show that intact spinach leaf mitochondria can oxidise glycine with good respiratory control and its oxidation is coupled to three phosphorylation sites. The conversion of glycine to serine is currently considered to be the major reaction releasing CO_2 during photorespiration at temperatures up to $25^\circ C$. An alternative reaction leading to the evolution of photorespiratory CO_2 is the direct decarboxylation of glyoxylate to formate, in both peroxisomes and chloroplasts, by a non-enzymatic reaction with H_2O_2 . It is thought unlikely, however,

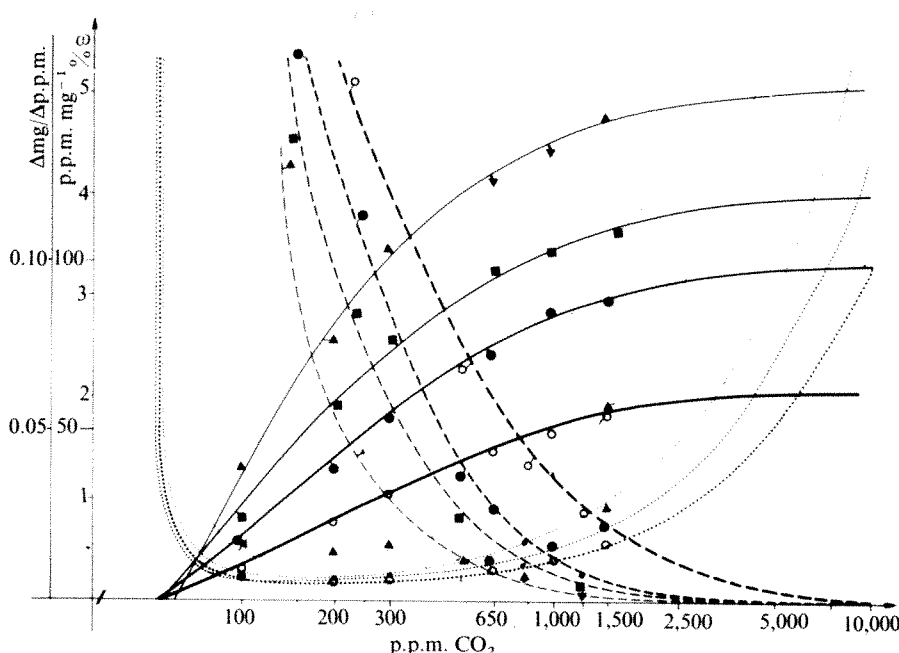


Fig. 2 The effect of CO_2 concentration at three temperatures ($\blacktriangle$, $15^\circ C$; $\blacksquare$, $25^\circ C$; $\bullet$, $35^\circ C$) on net photosynthesis at a fixed light intensity ($0.15 \text{ cal cm}^{-2} \text{ min}^{-1}$). Other symbols as in Fig. 1.

that this non-enzymatic decarboxylation in peroxisomes contributes more than 10–20% of the CO_2 released during photorespiration (see Halliwell *New Phytol.* 73, 1075; 1974).

The superoxide free radical anion (O_2^-) (produced by the reduction of O_2 in a one-electron step) may also play an important part in photorespiration. Although there is sufficient catalase present in the peroxisomes to destroy 80% or more of the H_2O_2 generated during glycolate oxidation, mitochondrial fractions from spinach leaves and purified

mitochondria from some etiolated tissues have been shown to generate H_2O_2 *in vitro*. The production of H_2O_2 by isolated chloroplasts is a well known phenomenon. Superoxide dismutases which catalyse the breakdown of O_2^- to O_2 and H_2O_2 have been found in the chloroplasts and mitochondria of spinach leaves. Since both mitochondria and chloroplasts contain systems which can generate O_2^- , it is likely that superoxide dismutases function to remove it. Their significance in relation to CO_2 release in photorespiration is, however, still to be evaluated. $\square$

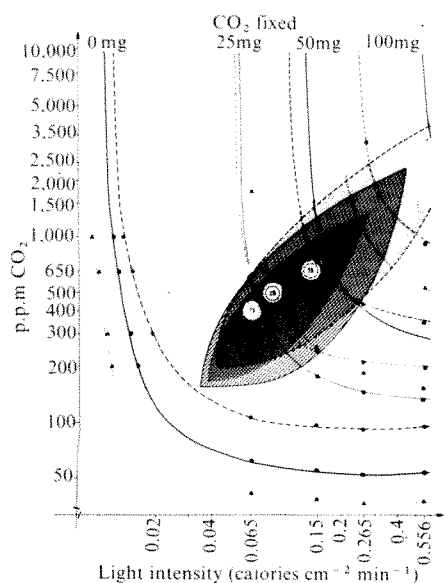


Fig. 3 Points of equal net photosynthesis at three temperatures and variable CO_2 concentration and light intensity. The figure depicts the points of optimal use of light intensity and CO_2 tension for maximal photosynthesis. Symbols as in Fig. 2.

Growth hormones and serum factors

from Robert Shields

It has been apparent for some time that only around 10% of the insulin-like activity in serum can be suppressed by insulin antibodies, but only now has the nature of the rest of the activity become clearer. Recent reports show that this non-suppressible insulin-like activity (NSILA) is due to a peptide structurally related to insulin, and that this peptide is probably the major growth hormone in the body as well as being a "serum factor" for *in vitro* cell culture.

Attempts to purify NSILA have been hampered by the fact that no organ seems to have high levels of

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activity and the concentration of the protein in plasma is very low. Industrial scale procedures are needed to isolate only a few milligrams of material from several tons of human plasma. The usual purification procedure begins with the fractionation of plasma by the Cohn procedure (essentially a series of controlled ethanol precipitations under increasingly acid conditions). Acid/ethanol precipitation of the Cohn IV precipitate (weighing a few kg) produced a small amount of soluble activity (NSILA-S) with most of the activity remaining in the precipitate (NSILA-P). It was thought therefore that this represented two distinct molecular species of molecular

weights 6,000 and 90,000 respectively. But it now seems likely that NSILA-P is an artefact generated by the acid/ethanol procedure¹, since with acid extraction alone 90% of the activity is recoverable in the soluble fraction².

Using the insulin-like effects of the material on adipose tissue as a guide, further purification was achieved by molecular sieve chromatography, preparative gel electrophoresis and ion exchange chromatography; finally the activity was isolated in two structurally similar peptides². Since the plasma used as starting material for the isolation of NSILA was pooled from several thousand individuals it is not clear whether these two peptides are the products of two genetic loci in an individual or are allelic variants in the population. Preliminary sequences of the N-terminal region of NSILA-I and NSILA-II reveal that the proteins are very similar to each other and that 50% of the first 30 residues of NSILA-I are identical to the 30 residues of the insulin B chain. This has led Rinderknecht and Humbel to propose that proinsulin may have evolved through gene duplication of an ancestral gene coding for a molecule very similar to NSILA³. It will be interesting to see if the remaining 20 residues of NSILA bear any resemblance to the C peptide of proinsulin which connects the N terminal B chain to the insulin A chain.

Role for NSILA

Many cells examined seemed to have membrane receptors for both insulin and NSILA^{4,5}. The sites are distinct but not surprisingly, in view of the homology of NSILA and insulin, high doses of one hormone will interact with the receptor for the other. This explains why high concentrations of insulin may mimic the effects of physiological levels of NSILA and *vice versa*^{6,7}. The physiological role of insulin is relatively well-understood, what then is the role of NSILA? It is obviously not active *in vivo* as an insulin analogue since diabetic animals have normal plasma levels of NSILA¹. It turns out that NSILA is probably

the major mediator of the effects of the classic trophic hormone, growth hormone.

Growth hormone and somatomedin

The role of growth hormone was determined historically by the observation that hypophysectomised animals fail to grow normally and that this defect can be largely repaired by injection of growth hormone (GH). But attempts to demonstrate the action of GH on skeletal tissue *in vitro* failed until it was realised that GH elicited the appearance of a second hormone that was responsible for many of its attributed physiological effects. This second hormone was originally termed sulphation factor since it was active in promoting sulphate uptake into cartilage. It is now clear however, that this is only one of its activities and amongst other activities it also possesses non-suppressible insulin-like activity. The factor was renamed somatomedin to imply that it mediated the effects of GH (somatotrophin)⁸.

Attempts to purify somatomedin from plasma proceeded on similar lines to those for NSILA, and the purification of a number of peptides with somatomedin (SM) activity has been reported. The biological assay used to guide purification was the incorporation of radioactive sulphate into cartilage, probably a very indirect assay for protein synthesis in the chondrocyte. Indeed, somatomedins have been shown to stimulate RNA and protein synthesis as well as other components of the pleiotypic response in skeletal tissue⁹. Two sources of cartilage are used to follow the purification of somatomedin activity, rat costal cartilage⁹ and chick pelvic leaflets¹⁰. Using the chick assay as a guide the isolation of two somatomedins has been described¹¹. Somatomedin A (SMA) is reported to be a neutral peptide active in the chick assay and somatomedin B (SMB) is an acidic peptide with no activity in the chick cartilage assay but is a growth factor for cultured cells¹². Using the rather more tedious and expensive rat cartilage assay another group of

workers have isolated a basic protein which they term somatomedin C (SMC)⁹. There is now an overwhelming body of evidence suggesting that SMC, SMA and NSILA purified by different groups are either very closely related, or most probably variants of the same molecule, whose active form in the plasma produces the effects of GH.

The probable relationship of SMA and NSILA is suggested by their similar purification schedules, their closely similar physicochemical properties and the fact that at no stage of their purification could insulin-like activity be dissociated from somatomedin activity¹³. Furthermore, these proteins bind to receptors with similar affinity and are displaced by high doses of insulin, and both activities are growth hormone dependent in that levels are low in pituitary dwarfs and high in acromegalics. Initially there was some doubt about the relationship of SMA to SMC and NSILA, as SMC and NSILA were only weakly active in the chick cartilage assay used to detect SMA¹⁴. However, the purified SMA peptide was itself hardly active in this assay although it was active in the rat cartilage system and so possessed SMC activity¹⁵. This puzzle was resolved when it was demonstrated that purified NSILA required the presence of another unidentified serum factor (not under GH control) in order to show sulphation factor activity in the chick assay¹⁶, although stimulation by NSILA of the pleiotypic response in chick cartilage was independent of this serum factor. Furthermore, SMA and SMC compete for the same receptor¹³. Definitive proof that SMA, SMC and NSILA are related must await comparative amino acid sequence data but the need for large amounts of pure material may mean that this proof may take some time.

Trophic action of NSILA

The trophic action of NSILA is not confined to chondrocytes; the factor is very active on cultured animal cells in promoting the parameters of the pleiotypic response and DNA synthesis^{2,17}. Insulin at pharmacological levels has often been used as a growth factor for cultured cells, and the reason for its effect can now be seen to be due to its cross reaction with NSILA receptors on the cell surface. In fact, physiological doses of NSILA have marked trophic effects in cell culture, so NSILA is probably an important member of the elusive serum factors and is quite possibly the one of major physiological relevance.

With so much NSILA in the plasma why aren't animals permanently hypoglycaemic? The answer seems to be that plasma contains a specific binding protein for NSILA which limits its

Table 1 Some of the properties of NSILA and somatomedin

Property	NSILA	SMA	SMB	SMC	Insulin (High doses)
Dependent on GH concentration	+	+	+	+	—
Carrier protein present in serum	+	+	—	+	—
Insulin-like activity in fat cells	+	+	—	+	±
Sulphation factor activity in chick cartilage*	±	±	—	—	±
Sulphation factor activity in rat cartilage	+	+	—	+	+
Growth promoting activity in cells <i>in vitro</i>	+	—	+	+	+
Competition with high doses of insulin for receptor	+	+	—	+	+

*Full activity in this assay probably requires the presence of another serum factor.

diffusion across capillaries into the extravascular space¹⁸. This helps to explain why the concentration of NSILA in lymph is half that in plasma¹⁹ whereas insulin (which has no binding protein) can equilibrate between these two compartments. The most intriguing feature of this binding protein is that its concentration in the plasma is regulated by GH^{20,21} and the binding of NSILA to its binding protein prolongs NSILA's half-life in plasma from a few minutes to several hours. The release of NSILA from its binding protein is thus a major factor in governing the availability of this growth promoting hormone to the tissues of the body. This also explains why searches in various organs for high concentrations of NSILA were unsuccessful; NSILA is concentrated in one of the largest 'organs' of the body—the plasma.

collaborators who find some homologies in peptide sequences between the cholera toxin and thyroid-stimulating hormone (TSH). Mullin *et al.* *Proc. natn. Acad. Sci. U.S.A.* **73**, 842, 1679; 1976; Ledley *et al.* *Biochem. biophys. Res. Commun.* **69**, 852; 1976; Meldolesi *et al.* *Proc. natn. Acad. Sci. U.S.A.* **73**, 4060; 1976). For this reason alone, current research on bacterial toxin structure and action has wide implications.

Several recent papers provide detailed information on the structure of tetanus and diphtheria toxins. These two toxins superficially have very similar structures and in the native state consist of single polypeptide chains which are easily 'nicked' by proteases into two polypeptides held together by disulphide bonds. Both polypeptide 'subunits' are essential for the activity of either toxin on intact cells. One of the fragments of 'nicked' diphtheria toxin (A subunit), however, is active in inhibiting protein synthesis in cell-free extracts by inactivating elongation factor 2 (translocase) in precisely the same way as intact toxin exerts its killing effect on unbroken cells. The second polypeptide subunit of nicked diphtheria or tetanus toxin then is reasonably assumed to be required to attach the toxin to the surface membrane of whole cells. Until recently this point was hard to establish for diphtheria toxin, since the B subunit is insoluble under normal conditions after disulphide bond cleavage of nicked toxin and may be kept in soluble form only in urea or other protein denaturants. Two groups have now devised methods for the isolation of stable B fragments. Helting and Zwisler (*Behring Inst. Mitt.* No. 59, 92–101, 1976) treated diphtheria toxin with formaldehyde under mild conditions before trypsinisation and reduction of disulphide bonds. The B fragment was immunogenic in rabbits or guinea pigs and the specific antibodies elicited by immunisation blocked the lethal action of diphtheria toxin in animals or whole cells, presumably by interfering with the interaction of the appropriate peptide sequence of the toxin with specific surface membrane receptors. In contrast, these antibodies failed completely to affect the enzymatic activity of isolated fragment A in inhibiting *in vitro* protein synthesis. More direct evidence for fragment B interactions with cells was obtained by Everse *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **74**, 472; 1976). The chains were stabilised either by using low concentrations of sodium dodecyl sulphate or by sulphonation. Solutions containing B fragment protected HeLa cells from the effect of intact diphtheria toxin, while A fragment was completely inactive. Although rather large

amounts of B fragment were required for protection against the toxin the effect does seem to be specific and strengthens the idea that the B fragment of the toxin molecule probably exerts the initial interaction with cell membrane receptors. Everse *et al.* also showed that 'unnicked' toxin was inactive when tested against HeLa cells under conditions where the 'nicked' toxin was highly cytotoxic. Probably, therefore, interaction of the toxin with cell surface receptors takes place most efficiently after 'nicking', a point that is interesting in the context of reports that some tumour cells are more sensitive to the action of diphtheria toxin than normal cells (Iglewski & Rittenberg *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2707; 1974; Pappenheimer & Randall *Proc. natn. Acad. Sci. U.S.A.* **72**, 3149; 1975) and the induction of proteolytic enzymes in some transformed cell lines (see for example Chen & Buchanan *Proc. natn. Acad. Sci. U.S.A.* **72**, 1132; 1975).

The subunit of 'nicked' tetanus toxin analogous to fragment B of diphtheria toxin has also been identified recently (Helting & Zwisler *J. biol. Chem.* **252**, 187; 1977; Helting *et al. J. biol. Chem.* **252**, 194; 1977). The larger of the two polypeptides (heavy chain) derived after mild trypsinisation retained all of the receptor binding activity of the native toxin and a specific antiserum directed against a part of the heavy chain completely prevented activity of whole toxin. These new techniques, therefore, make available stable fragments of both diphtheria and tetanus toxin that are efficient in eliciting antibodies which interfere with the interactions with brain receptors and neutralise toxic action. Such vaccines may have advantages over the standard preparations against inactivated toxoids. Reactions of delayed hypersensitivity are known in immunisation, particularly of older children, for example (see Helting & Zwisler *op. cit.* 1976) and the use of peptide fragments lacking the antigenic determinants producing these undesirable responses would be a distinct advance. □

Toxin subunits

from a Correspondent

DURING the past couple of years knowledge of the mechanism of action of bacterial toxins, particularly cholera, tetanus and diphtheria toxins, and studies of hormone action have pointed out several areas of similarity in these diverse biological systems. Apparently, similar surface membrane receptors may be part of the systems responsible for uptake of some toxins and hormones, an obligatory step in the biological action of these substances on cells. Perhaps the most striking result comes from L. D. Kohn and his

Microtubes

from Peter J. Smith

EVERY now and then someone decides to investigate a terrestrial phenomenon which has been known for many decades, if not a hundred years or more, but which has never before been studied in any detail. Quite why certain phenomena should be ignored for so long is far from clear. Some have probably been considered trivial—of interest in themselves, perhaps,

- ¹ Froesch, E. R. *et al.* in *Advances in Metabolic Disorders* (eds Luft & Hall) **8**, 203 (Academic, New York, 1975).
- ² Rinderknecht, E. & Humbel, R. E. *Proc. natn. Acad. Sci. U.S.A.*, **73**, 2365 (1976).
- ³ Rinderknecht, E. & Humbel, R. E. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4379 (1976).
- ⁴ Megyesi, K. *et al. J. biol. Chem.* **250**, 8990 (1975).
- ⁵ Zapf, J. *et al. Arch. Biochem. Biophys.* **168**, 630 (1975).
- ⁶ Morell, B. & Froesch, E. R. *Eur. J. clin. Invest.* **3**, 119 (1973).
- ⁷ Oelz, O. *et al. Eur. J. clin. Invest.* **1**, 48 (1970).
- ⁸ Daughaday, W. H. *et al. Nature* **235**, 107 (1972).
- ⁹ Van Wyk, J. *et al. in Recent Progress in Hormone Research* **30**, 259 (Academic, New York, 1974).
- ¹⁰ Hall, K. *Acta endocr.* **63**, 338 (1970).
- ¹¹ Uthne, K. *Acta endocr. Suppl.* **175**, 1 (1973).
- ¹² Westermark, B. *et al. Expl. Cell Res.* **96**, 58 (1975).
- ¹³ Hall, K. *et al. in Advances in Metabolic Disorders* (eds Luft & Hall) **8**, 19 (Academic, New York, 1975).
- ¹⁴ Sievertsson, H. *et al. in Advances in Metabolic Disorders* (eds Luft & Hall) **8**, 47 (Academic, New York, 1975).
- ¹⁵ Van Wyk, J. *et al. in Advances in Metabolic Disorders* (eds Luft & Hall) **8**, 127 (Academic, New York, 1975).
- ¹⁶ Froesch, E. R. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2904 (1976).
- ¹⁷ Morrell, B. & Froesch, E. R. *Eur. J. clin. Invest.* **3**, 119 (1973).
- ¹⁸ Zapf, J. *et al. Arch. Biochem. Biophys.* **168**, 638 (1975).
- ¹⁹ Cohen, K. L. & Nissley, J. P. *Endocrinology* **97**, 654 (1975).
- ²⁰ Cohen, K. L. & Nissley, J. P. *Acta endocr.* **83**, 243 (1976).
- ²¹ Moses, A. C. *et al. Nature* **263**, 137 (1976).
- ²² Zingg, A. E. & Froesch, E. R. *Diabetologia* **9**, 472 (1973).
- ²³ Takano, K. *et al. Acta endocr.* **80**, 14 (1975).
- ²⁴ Yalow, R. S. *et al. in Advances in Metabolic Disorders* (eds Luft & Hall) **8**, 73 (Academic, New York, 1975).

but with no potential for influencing other branches of the Earth sciences and thus of no significance to the geological mainstream. But not all examples of scientific neglect can be explained away so easily. Consider, for instance, the microscopic tubes, or microtubes, in igneous rocks. There must be a reason for their presence; and an obvious case can be made for saying that they should be able to provide valuable information on the history of their hosts. Yet unlike primary and secondary inclusions in rocks, about which there is now a vast literature, they have been almost completely overlooked.

Richter and Simmons (*Earth planet. Sci. Lett.* **34**, 1; 1977), who have begun a detailed study of microtubes, define them as cavities of circular or elliptical cross section and having diameters generally less than $5\text{ }\mu\text{m}$ and lengths greater than $10\text{ }\mu\text{m}$. They may be hollow or partly or completely filled with a solid phase, but they are distinct from the better-known euhedral needle-like inclusions of minerals such as rutile and apatite. They are also distinct from microcracks, which have one dimension (generally less than a few micrometres) much smaller than the other two (greater than several tens of micrometres). They do not have faceted walls and so can be neither negative crystal forms of the host grain nor the result of acicular inclusions. Yet they are evidently single crystal phenomena, for they never cross grain boundaries.

The first known reference to microtubes was by Sorby (*Q. Jl Geol. Soc.* **14**, 453; 1858) who recognised several isolated examples in vein quartz but made little comment about them. Then Rosenbusch (*Mikroskopische Physiographie der Massigen Gesteine*, Stuttgart, 1907) found very fine cylindrical canals in samples of deformed granite, concluding that they were formed by stresses incurred during mountain building; and Honess (*The Nature, Origin and Interpretation of Etch Figures on Crystals*, New York, 1927) later discovered tubular etch pits in natural quartz. More recently only a handful of workers have mentioned the presence of microtubes and even fewer have commented on them more than briefly, thereby giving the impression that such features are rare. Yet they are very far from rare. They may have been overlooked by many petrographers; but Richter and Simmons have observed them in granite, quartz monzonite, syenite, granodiorite, anorthosite, diabase, gabbro and even lunar basalt, the minerals involved being quartz, K-feldspar,

plagioclase, muscovite, biotite, pyroxene and olivine.

So microtubes are evidently more common than previously supposed. But how do they form? Richter and Simmons conclude from petrographic and scanning electron microscope studies that there is no single process of formation but at least three. It is clear from their groupings, associations and spatial relationships that many microtubes arise from the partial annealing of microcracks. Others, however, appear to have resulted from the etching of crystal defects such as dislocations by naturally occurring fluids, whilst still others are evidently primary features produced by the inclusion of fluid material during crystal growth.

There may be other, as yet undiscovered, ways in which microtubes

form. But such diversity is an advantage, for it suggests even more strongly that the study of microtubes may throw light on the various physical and chemical conditions to which many igneous rocks may be subject both during and subsequent to their formation. Microtubes resulting from annealing might give information on a rock's deformation history, etch tubes could possibly provide evidence of the types of fluid that have circulated through a rock; and primary tubes should provide clues to the conditions obtaining during crystal growth. For the time being Richter and Simmons pursue these possibilities no further; but it is clear from the progress they have made so far that it may have been a mistake to have given microtubes so little attention in the past.

Sex ratios in social insects

from John Krebs

IN a paper published just over a year ago, R. L. Trivers and H. Hare (*Science* **191**, 249; 1976; see also Krebs & May *Nature* **260**, 9; 1976) reported results which in one fell swoop appeared to verify Fisher's theory of the sex ratio, Hamilton's kinship theory of altruism in social hymenoptera, and Trivers's own ideas on manipulation of parents by offspring; a virtuoso display indeed! This seminal and apparently incontrovertible piece of work has now come under vigorous attack from R. D. Alexander and P. W. Sherman (*Science* **196**, 494; 1977).

To recap on the previous story, Trivers and Hare accounted for the apparent altruism of sterile female workers of social hymenoptera such as bees and ants in the following way. As a result of the sex determination system, in which haploid males develop from unfertilised eggs, the diploid females share $3/4$ of their genes by direct descent with their full sisters, and $1/4$ with full brothers. The diploid mothers share half their genes with sons and daughters alike. As W. D. Hamilton first showed, this state of affairs means that a female who has the "choice" between producing her own daughters or rearing reproductive younger sisters makes a genetic profit by doing the latter. However, in order to explain fully the evolution of sterile worker castes in terms of Hamilton's kin selection theory, one has to consider why the workers also rear brothers (with whom they share only $1/4$ of their genes). Trivers and Hare, following up a suggestion of Hamilton, pointed out that workers should be

more prone to rear reproductive sisters than drones. More precisely, they should invest three times as much effort in rearing new queens. If drones are only a third as common as queens they will have on average three times the mating success, which as far as a worker is concerned, exactly compensates for the threefold difference in relatedness. In any typical diploid outbreeding species, the mother's optimal ratio of investment in daughters and sons is 50:50. (This is the only stable equilibrium since the rarer sex is always at an advantage.) Trivers's and Hare's striking finding was that the ratio of investment in 21 species of monogynous (one active queen per colony) ants was close to 3:1 in favour of females, just as predicted by the simplest genetic model based on the assumption of worker success in manipulating the sex ratio to their advantage. As a 'control', Trivers and Hare showed that the investment ratio was much closer to 1:1 in slave-making ants and non-social hymenoptera where the queen presumably achieves her optimum. Far from the picture of selfless worker altruism, these remarkable results suggest that the queen is, in effect, farmed by the workers as a reproduction machine.

Alexander and Sherman question not only the factual basis and statistical treatment of Trivers's and Hare's evidence but also point to an alternative interpretation. The critique is detailed and complex and I can only highlight a few points. Three main

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challenges to the factual evidence emerge: multiple matings are commoner than Trivers and Hare assume; workers often lay eggs which develop into drones; and the investment ratio in slave-making ants, termites, and non-social bees and wasps seems to be slightly biased towards females.

The calculations of degree of relatedness on which Trivers's and Hare's predictions are based depend critically on their assumption that the workers and the reproductive females these rear are full sisters. If the queen mated with three males and mixed the sperm at random, the whole argument for a bias on the part of workers would collapse. Alexander and Sherman point out that multiple matings are quite common in social hymenoptera. But the problem is that single matings are virtually impossible to prove even if they are common. One might also add that the mating habits of present-day queens are probably less critical to Trivers's and Hare's argument than those of queens during the early evolution of eusociality: studies of mating in the semisocial hymenoptera suggest that monogamy may have been quite common at this intermediate evolutionary stage.

The kinship theory of social hymenoptera does in fact predict that workers should attempt to lay eggs, since by so doing they substitute sons (degree of relatedness $1/2$) for brothers (to whom they are related by only $1/4$). This point was discussed at length by Trivers and Hare, who consider egg laying to be an alternative strategy to manipulating the investment ratio by workers. The only point in question is whether the monogynous ants selected for analysis by Trivers and Hare are species in which the sex ratio is influenced by laying workers, a point on which there seems to be no concrete evidence.

Alexander's and Sherman's doubts concerning the claims of Trivers and Hare that the ratio of investment in slave-making ants, termites, and non-social hymenoptera is close to 1:1 stem from the fact that the original data are incredibly variable, some nests showing a strong female bias and others a trend towards males. Alexander and Sherman conclude that the termites show a slight female bias (kinship theory predicts no bias) and that some of the data for unsocial bees and wasps used by Trivers and Hare may be subject to the sampling bias. Nevertheless they admit that the basic trend—a stronger female bias in investment ratio in monogynous ants than in non-social hymenoptera—is still valid.

Much more important than these hair-splitting criticisms, is Alexander's and Sherman's suggestion that a plausible alternative hypothesis could

account for the results obtained by Trivers and Hare. Interestingly, this alternative idea was also developed by W. D. Hamilton, who pointed out that Fisher's theory of equilibrium sex ratios does not hold when there is inbreeding and siblings compete with each other for matings (as males usually compete for females rather than *vice versa*, the argument can be taken to refer to competition between brothers). Suppose, for example, that a female's daughters are all fertilised by their brothers. The mother's best strategy is to produce just enough males to ensure that all daughters are fertilised, putting most of her resources into female offspring. Fisher's 1:1 equilibrium no longer holds, because the expected reproductive success of sons does not depend on what goes on in the rest of the population. Whenever this sort of competition between brothers occurs the optimal sex ratio will be biased towards females, the exact bias depending on the degree of inbreeding.

If, as Alexander and Sherman suggest, inbreeding and sibling competition for mates is common in social hymenoptera, the biased investment ratio might be optimal for the queen and not, as Trivers and Hare suggest, for the workers, who would like a still greater bias in favour of females. The two hypotheses, both apparently consistent with the data, are not necessarily mutually exclusive. The mean investment ratio for the monogynous ants analysed by Trivers and Hare was about 3.5, slightly in excess of the predicted 3.0. This could conceivably be an effect of both mating competition and worker manipulation, although one cannot check this as Alexander and Sherman are unable to predict an optimal investment ratio for the queen without more information on the extent of mating competition.

Trivers's and Hare's hypothesis, on the other hand, not only predicts with reasonable success (given the difficulty of measuring 'investment') the observed bias in monogynous ants, but also predicts the difference in investment ratio between monogynous ants on the one hand, and the slave-making ants, non-social bees and termites on the other. In order to account for this difference, Alexander and Sherman have to invoke the (plausible) argument that inbreeding is less common in the second group than in the first. (They also point out that among the non-social hymenoptera, a slight female bias in the sex ratio is more often observed in those species which do have inbreeding).

To sum up, although the evidence is not as clear cut as it first appeared, Trivers's and Hare's hypothesis still provides the more parsimonious pre-

dictive explanation of the data, and what is more, it also predicts other observations, for instance laying by workers, and the absence of sterile males in the hymenoptera but not in the termites. Nevertheless the sex ratio is almost certainly modified to some extent by inbreeding. □

Low temperature electron microscopy

from Patrick Echlin

The 1st International Meeting on Low Temperature Biological Microscopy was held in Cambridge 4-7 April, 1977 under the auspices of the Royal Microscopical Society. Edited versions of all the papers will appear later this year in the *Journal of Microscopy*.

ALTHOUGH low temperature techniques are now assuming a greater significance in biological investigations, many biologists are unhappily unaware of the complex physicochemical properties of ice and the nature of the freezing process in cells and tissues. This interdisciplinary meeting sought to redress this situation by bringing together ice physicists, physical chemists, cryobiologists, engineers and microscopists to see whether these techniques are indeed fulfilling the promise of perfect preservation of ultrastructure and functional physiology. All the participants overtly or tacitly accepted the dictum that 'cold is good' but there was extensive discussion as to what constitutes 'good' in relation to ultrastructure, analysis and vitality. J. Farrant (Clinical Research Centre, Harrow) showed that with a few exceptions good ultrastructure, retention of soluble constituents and preservation of vitality were probably always likely to be mutually exclusive. This, the cryobiologist's trinity, could only be achieved by vitrification, a process rarely achieved in biological tissues.

Various techniques which would help overcome the problems associated with ice crystal damage were discussed. H. Sitte (University of Homburg-Saar), H. Muller (Technical High School, Zurich), W. Bald (University of Oxford) and L. Seveus (Stockholm) described new fast-freezing techniques, all of which could theoretically give sufficiently fast cooling velocities in small pieces of biological material to bring about a shift from heterogeneous nucleation to homogenous nucleation with the consequent formation of very small ice crystals. Alter-

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native approaches were offered by T. Nei (University of Sapporo, Japan), H. Skaer (University of Cambridge) and A. Walter (Clinical Research Centre, Harrow) who advocated the use of penetrating and non-penetrating cryoprotectants. Nei showed images of remarkable preservation in red blood cells frozen and thawed in the presence of 20% glycerol, and Skaer gave details of some high molecular weight polymers which although they do not enter cells, limit the size of intracellular ice crystals to nanometre dimensions even in pieces of tissue 1 mm in diameter. Such polymers have important implications in the preparation of tissues for freeze fracture and X-ray microanalytical studies which are best carried out on chemically unperturbed tissues.

There was extensive discussion on the process of sectioning and freeze fracturing and the nature and extent of the artefacts associated with these techniques. A. Saubermann (Harvard Medical School) demonstrated that the process of cutting frozen material is a mixture of sectioning and multiple fracturing depending on temperature, cutting speed and knife angle, and U. Sleytr (Vienna) and T. Robards (University of York) showed that plastic deformation can occur at temperatures well below the glass transformation point of ice. Sufficient energy is released during cleavage to deform biological material and great care must be taken when interpreting freeze fracture images. The same point was taken up by D. Branton (Harvard University) and S. Bullivant (University of Auckland) who showed that some of the artefacts associated with membrane structure are produced either during specimen pretreatment and freezing or simply by the collapse of the lipid layers after membrane cleavage.

Low temperature techniques are clearly no longer the preserve of the morphologists and a number of papers were given showing how both frozen-hydrated and frozen-dried tissues and sections could be used for the analysis of diffusible ions and electrolytes. K. Zierold (Max Planck Institute, Dortmund) and M. Sjostrom (University of Umea, Sweden) gave details of low temperature X-ray analytical studies on mammalian muscle. T. Barnard (Wenner-Gren Institute, Stockholm) related the diffusion of electrolytes in frozen sections of adipose tissue to changes in the electron density of the sections. One of the main problems of low temperature microscopy is to ensure that the biological material stays cold enough during preparation and examination. P. Echlin (University of Cambridge) considered these problems in relation to cryofractured frozen bulk samples and sections examined at

low temperatures in the scanning electron microscope and suggested a number of criteria which could be used to judge the hydration state of material in the microscope. Such problems are less exacting for the smaller samples examined by K. Taylor (MRC Laboratory of Molecular Biology, Cambridge) in the transmission microscope who used the disappearance of the ice diffraction pattern as an indicator of specimen hydration in catalase crystals and bacterial cell walls. There were close similarities between the negative stain images of catalase and the hydrated crystals, and it looked as if the ice was acting rather like a negative stain. The earlier hopes that low temperatures might dramatically diminish radiation damage in high resolution images were dashed when R. Glaeser (University of California, Berkeley) showed that depending on the specimen, the damage is only lessened by a factor of two when operating at liquid nitrogen temperatures and a factor of five when operating only a few degrees above absolute zero.

There was considerable discussion on the interpretation of morphological data obtained from frozen sections and fractures. T. Gulik-Krzywicki (CNRS Gif-sur-Yvette) showed that it is possible to correlate low temperature X-ray diffraction patterns with images obtained by freeze fracture electron microscopy, and H. Chanzy (CNRS Grenoble) described a technique for low temperature electron diffraction. Whereas these specialised techniques and the use of image processing can help with the interpretation of the inert freeze fracture images, such methods are of little use for unstained frozen sections which give images containing few of the subcellular features recognisable from conventional microscopy. Cryohistologists are going to have to re-educate themselves to recognise images in material produced and examined at low temperatures. □

Head-on proton collisions

from Peter Landshoff

MOST of the information on the structure of protons has been obtained by bombarding them with very high energy electrons, and these are the experiments that have provided strong, though indirect, evidence that protons contain quarks. The quarks apparently

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A hundred years ago

THE Superintendent's Report on the Botanic Garden and Public Plantations for 1875-76 has recently been officially published in Jamaica. It deals almost entirely with plants of economic value, foremost of which is the coffee, the ordinary kind (*Coffea arabica*), apparently giving way to its formidable rival *Coffea liberica*, which was introduced to Jamaica in 1874, and is now thriving, especially in some districts. In one situation, at a height of about 1,000 feet above the sea, a plant that had only been planted out a little over a year has already fruited. This seems to indicate that in the course of a few years the new coffee may be widely cultivated in Jamaica from plants raised from seeds ripened in the island.

From *Nature* 16, 24 May, 73; 1877.

have very small diameters, or are maybe even pointlike, although the proton as a whole has a more diffuse structure in which the quarks are embedded. The nature of the remaining constituents is not yet clear. Certainly they include quark-antiquark pairs, some of which may be bound tightly together to form mesons. Very possibly they include also the 'gluons' that bind quarks and antiquarks together in the quantum field theories that mathematical physicists are constructing in attempts to unify the various fundamental forces of nature.

The evidence that there are quarks inside the proton is indirect, because no free quarks have yet been seen. Indeed, many physicists believe that it may not be possible for quarks to be knocked out of their parent protons. (There have been various attempts to construct such theories of 'quark confinement'.) For this reason, it is desirable to verify the quark structure in other types of experiment. Beams of high-energy muons and neutrinos have also been used to probe the proton, and the information that they give, while again indirect, seems to confirm and complement that which comes from electron scattering. A quark structure in the proton seems rather sure.

Electrons, muons and neutrinos are particularly useful as probes because their own structure is especially simple and well understood. However, additional information can be obtained by making pairs of protons collide at very high energy.

The diameter of a proton is about 10^{-12} m—much greater than that of a quark, but infinitesimal compared with the width of a laboratory proton beam. So when two high energy beams are fired at each other, as in the Intersecting Storage Rings of the European laboratory at CERN, most of the protons do not collide. Those that do nearly always are in glancing collision. The dynamics of such collisions, where only the outer parts of the two protons interact, is complicated. It does not involve the quark structure in a very direct way, and the particles produced in the interaction (mostly pions) are swept along more or less in the initial directions of the two colliding beams.

Very rarely, two protons collide head-on and in this case their quark structure may be expected to be involved rather directly. Also, the head-on collisions are quite likely to produce particles that emerge with a fast sideways motion relative to the initial beams. Although these 'large-transverse-momentum' events are comparatively very rare, they can readily be identified, and their study has been an important part of the CERN Intersecting Storage Rings research programme during the past four or five years. Some important information has come also from experiments at Fermilab in the United States.

High-energy proton-proton collisions produce a large number of additional particles, making the analysis of the interesting events complicated. So far the analysis cannot be complete because of the limitations of the available particle detectors. However, several of the CERN experiments have recently observed an interesting "jet structure" in the large-transverse-momentum events: the sideways-moving particles emerge together in more or less the same direction, so as to form a jet of particles. This jet structure has been seen most clearly in three experiments using the CERN Split Field Magnet facility. These experiments were performed by a group from CERN, by a CERN-College de France-Heidelberg-Karlsruhe collaboration, and by a British-Scandinavian-French collaboration. More recently, a collaboration from Caltech, UCLA, Fermilab, Chicago and Indiana has reported results from a jet study at Fermilab. The total momentum of the two protons that collide is zero, so there are two jets, one emerging to each side of the initial beam direction (see Fig. 1). In addition to the two jets, several particles are produced that have small transverse momentum, as in the ordinary, glancing collisions.

Pairs of jets are known also to be a feature of collisions of high-energy electrons with positrons. In these col-

Transcriptional control of fertility

from J. R. Saunders

A RECENT paper from Neil Willetts (*J. molec. Biol.* **112**, 141; 1977) provides the first biochemical evidence that the fertility of bacterial plasmids is regulated at the level of transcription. Most of the genes specifying the ability to conjugate in *Escherichia coli* comprise a single 'transfer operon' which is present on the F factor and F-like plasmids. This operon consists of genes necessary for the production of sex pili, for surface exclusion and for the metabolism of DNA during transfer. It is transcribed in the order *traA*, *traL*, *traE*, *traK*, *traC*, *traF*, *traH*, *traG*, *traS*, *traD* (Helmuth & Achtman *Nature* **257**, 652; 1975). A further gene *traJ*, has a positive controlling function over the entire operon but lies outside it. In turn the expression of *traJ* is itself subject to negative control imposed by two further plasmid genes *finO* and *finP* acting in concert. F-like plasmids isolated from the wild generally transfer at a relatively low frequency due to this control. F itself is atypical in that it is a naturally occurring *finO*⁻ mutant and consequently transfers itself at a high rate. However F will recognise the *finO* product specified by other F-like plasmids and if present in the same cell such plasmids inhibit F fertility producing the *Fin*⁺ (*fi*⁺) phenotype.

Genetic tests have established that the gene products of the transfer operon are absent during *FinOP* fertility inhibition. Hitherto it was unclear whether the *traJ* product is necessary for transcription or translation of the operon. Willetts has distinguished between these possibilities by first constructing bacteriophage λ transducing phages carrying either *traJ* or the *traL*, *traE*, *traK* region. He then used DNA from these phages as probes for the synthesis of specific messenger RNA during *FinOP* transfer inhibition. His hybridisation experiments indicate that whilst mRNA homologous with this '*tra*' DNA is present in donor strains carrying F alone it cannot be found during transfer inhibition of F by the F-like R plasmid R100. This suggests that the transcription of *traJ* is repressed under the latter conditions. In the absence of the *traJ* product, transcription of the transfer operon itself is either not initiated or possibly terminated prematurely shortly after initiation. It should prove possible now to extend these studies on the expression of sex factors by utilising λ *tra* phages carrying further transfer regions of F and other plasmids.

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lisions, the electron and the positron annihilate each other. There is convincing evidence that the energy that they leave behind subsequently materialises as a quark and an anti-quark, which apparently do not escape as free particles. Exactly what happens is not understood, but first they "dress" themselves somehow by interacting with each other, so that they then emerge as two jets of ordinary particles. There are also indications that similar jets result from the collisions of high-energy electrons, muons or neutrinos on protons.

Because there is good evidence that

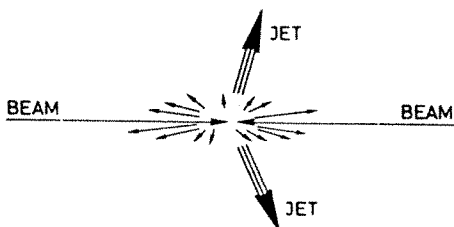


Fig. 1 The two jets produced in large-transverse-momentum collisions together with particles that have small transverse momentum relative to the incoming beams.

these jets originate from quarks, a natural guess is that the same may be true of the jets produced in the large-transverse-momentum proton-proton collisions. In the simplest model for this reaction, a quark from one of the protons scatters a quark from the other through a wide angle. The two quarks then somehow dress themselves, and result in the two jets. The remaining constituents of the two protons are responsible for the production of the longitudinally-moving particles.

There are other models that differ in detail. The experimental and theoretical question of whether the jets seen in proton-proton collisions do have the same character as those in electron-positron annihilation has yet to be answered. But it is already apparent that the large-transverse-momentum proton-proton reactions have fairly simple features which, in a way whose details are still to be determined, do reflect the quark structure of the proton. When the dynamics of these reactions are better understood, they will provide a unique way of investigating how quarks scatter on each other at high energies.

articles

Magma mixing: a mechanism for triggering acid explosive eruptions

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Injection of basic magma into acid magma causes superheating of the acid magma and vigorous convection. Vesiculation induced by convection and increased magma pressure fractures the volcanic edifice triggering an explosive acid eruption. The 1875 plinian eruption of Askja, Iceland is an example of an explosive eruption triggered by magma mixing.

THERE are many possible mechanisms of triggering explosive volcanic eruptions of acid magma. Most of these involve factors which ultimately lead to supersaturation of the melt with respect to volatiles¹. We have, however, been impressed by a number of examples of plinian pumice fall deposits where there is evidence that basic magma was intruded into acid magma and the two magmas were physically mixed by vigorous convection shortly before eruption. We will now describe this evidence and demonstrate theoretically that a number of effects of mixing are highly conducive to triggering an eruption. We propose that magma mixing is a common mechanism of triggering explosive eruptions of acid magma, although we emphasise that not all magmas that mix inevitably erupt, nor are all explosive eruptions caused by mixing.

Geological evidence

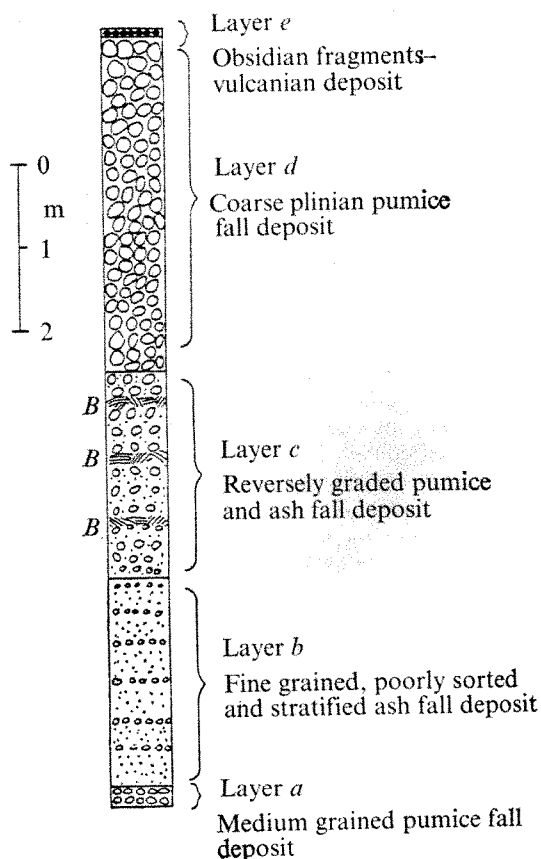
Tephra containing an intimate mixture of two or more contrasted compositions of magma is a common feature of acidic pyroclastic deposits and in our view the few examples described in the literature do not adequately reflect the abundance of mixed tephra occurrences²⁻⁶. In the cases observed by us the more basic component occurs either as bands and streaks or as irregular, rounded clots and inclusions in the acid pumice. The basic component is usually a volumetrically minor component, but shows evidence of its molten condition at the time of eruption by the occurrences of vesicles, flowage structures and its glassy condition. We now describe detailed case histories of explosive ejecta which shows these features from Askja, Iceland and Santorini, Greece.

Fig. 1 shows the stratigraphy and lithological characteristics of the mixed ejecta of the 1875 explosive eruption in Askja, Iceland. The layers formed over a period of 8.5 hours on the night of the 28-29 March 1875 (ref. 7). The plinian deposit (layer d) formed in six of those hours and represents 80% of the ejecta by volume. All the tephra layers show evidence of mixing and simultaneous eruption of basalt and rhyolite magma.

We have analysed the two end-member glasses which are found intimately associated in the 1875 ejecta (Table 1; analyses a and b). The basaltic component is a dark brown glass, poor in

crystal, and is similar in composition to the tholeiites which characterise the Northeastern Rift Zone of Iceland⁸. The basaltic glass occurs as irregular inclusions and streaks in rhyolitic obsidian and pumice and occasionally in bombs. More often it is found in separated, rounded, mixed dark scoria fragments. During most of the eruption the scoria clasts were finer than 1.0 cm and hence are progressively enriched in the tephra away from the source where most of the tephra less than 1 cm was deposited. Layer c (Fig. 1) grades laterally into a densely welded air fall tuff where both the

Fig. 1 Schematic section of the 1875 Askja tephra showing the principal lithological characteristics of the five layers. The thicknesses are the maximum observed for each layer on the north-eastern and eastern rims of Oskjuvatn. B refers to base surge horizons.



rhyolitic pumice and basic clasts have been deformed into *flamme*, demonstrating the molten condition of both magmas. During the closing stages of eruption the size of mixed ejecta markedly increased. Large spatter bombs and mixed bombs up to 5 m diameter containing both magmas were ejected.

The rhyolitic component contains less than 1% plagioclase and clinopyroxene crystals and is relatively low in total alkalis and high in CaO and MgO (Table 1) compared to other Icelandic rhyolites⁹. Some plagioclase phenocrysts are rounded but they contain glass inclusions of rhyolitic composition showing that they formed in equilibrium with the acid liquid. The rounding could be interpreted as resorption due to superheating of the rhyolite. Rounded grey clots (0.1–1.0 cm) are found as inclusions in the rhyolitic pumice in all the layers. These inclusions contain between 40% and 50% of plagioclase and pyroxene microphenocrysts set in a pale brown glass. The detailed petrological and volcanological relationships are complex, but are not pertinent to the present considerations. We merely wish to document the highly heterogeneous nature of the ejecta.

Table 1 Analyses of mixed tephra

	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>
SiO ₂	73.72	50.98	69.61	54.1
Al ₂ O ₃	12.92	13.81	14.14	16.6
TiO ₂	0.83	1.89	0.38	0.8
FeO	3.12	13.31	2.89	8.2
MgO	0.57	6.18	0.36	3.3
CaO	2.14	10.82	2.00	8.0
MnO	0.07	0.30	0.08	0.1
Na ₂ O	3.59	2.47	4.78	5.0
K ₂ O	2.53	0.30	3.07	1.3
P ₂ O ₅	0.12	0.21	0.07	—
H ₂ O	—	—	3.49	2.4
Total	99.61	100.27	100.87	99.8

a. Rhyolitic glassy *flamme* from welded part of layer *c*, Askja (average of 7 analyses); *b*. Basaltic glass, Askja (average of 5 analyses); *c*. Rhyodacite pumice, Santorini; *d*. Basaltic andesite scoria, Santorini. Analyses *a* and *b* were made on the electron microprobe. Analyses *c* and *d* are X.R.F. whole rock analyses (analyst A. Bond).

On the volcano Santorini several of the major acid plinian pumice fall deposits exposed in the caldera walls are mixed. Bond and Sparks¹⁰ recognised the following sequence of products in the Bronze Age Minoan eruption: plinian pumice fall deposit, base surge deposits, mud-flow deposits and ignimbrite. The bulk of the ejecta is rhyodacite containing 10–12% phenocrysts and there is no marked major element variation throughout the sequence. However, the upper half of the plinian deposit and the lower half of the base surge deposits contain vesicular, grey rounded pumice clasts (0.1–5 cm diameter) of basaltic andesite bulk composition (Table 1: analyses 3 and 4), which contain 25 to 35% phenocrysts set in a pale vesicular glass containing abundant quench crystals. The two types are quite distinct (SiO₂ 69.6 and 54.1%) and occur together in the same pumice clast. The tephra in the ignimbrite and lower part of the plinian deposit is not mixed.

Similar relationships are observed in the thick, prehistoric rhyodacitic pumice deposit (Bu) of the Middle Pumices Series¹¹. This deposit has two fall units, the lower one containing white rhyodacitic pumice only. In the upper fall unit grey, olivine bearing basaltic andesite pumice clasts, similar to the Minoan examples, are found intimately mixed with white rhyodacitic pumice. The proportion of basic tephra increases markedly upwards reaching an estimated 30% at the top of the upper fall unit. There are at least 12 acid pumice fall deposits exposed in the caldera walls of Santorini, which we have examined. Seven of these show field evidence of a mixed nature, with

relations between the two components similar to those described in the Minoan and Bu pumice deposits. The more basic component is not necessarily basaltic or indeed greatly different from the acid component: in one deposit the two components have SiO₂ contents of 61.1 and 65.7% respectively.

In these examples from Santorini and Askja mixing of the magmas can only have occurred shortly before eruption. In all cases the basic clasts are still dominantly glassy even in inclusions of a few millimetres size. Such inclusions could not survive in their acid host for any substantial time without either completely crystallising or dissolving by diffusion. In the case of Santorini, radiocarbon dates¹¹ indicate that periods between explosive acid eruptions are of the order 10⁴ yr. The mixing of the magmas can be shown, however, to have occurred no more than a few years before eruption and we believe that periods of weeks are more realistic for the time interval between mixing and eruption.

The interdiffusion of ions between two miscible silicate glasses can be estimated from:

$$l = \beta \sqrt{(Dt)} \quad (1)$$

where *l* is the length to which a diffusional wave penetrates from one area to another in a time *t*, *D* is the diffusion coefficient, and β is a constant which is conveniently 1.0 for 95% of the final equilibrium concentration¹². In the case of a sphere of basaltic glass, the length *l* may be set equal to the radius; for a radius 0.1 cm and for $D = 10^{-9} \text{ cm}^2 \text{ s}^{-1}$, which is typical of ionic diffusion rates at high temperature in silicate melts¹³, *t* is 3.85 months. In the Minoan deposits and Askja deposit, inclusions of basic material of 0.1 cm show little evidence of substantial chemical exchange with their host. We believe that the magmas were intermingled physically, but were erupted before any substantial chemical interaction took place. On Santorini we have observed the same relationships in several deposits and the eruptions clearly coincide with mixing events.

Model for magma mixing

We propose that magma mixing is a process which is likely to trigger an explosive eruption and that the features we have described in Askja and Santorini fit such a model. An acid magma emplaced in the upper crust is a slow moving and slow cooling body. Changes in physical conditions such as temperature and gas pressure will occur gradually, as crystallisation and conductive heat loss proceed, over periods of 10³–10⁵ yr (ref. 14). Gradients of temperature and physical properties will be small and rates of change slow. Intrusion of basic magma into the base of an acid magma chamber would dramatically alter this situation and produce an unstable, non-equilibrium system.

Studies of intrusive net-vein complexes³ show that the basic magma often forms pillow-like accumulations when intruded into acid magma. The temperature contrast between basaltic magma (1,100–1,300 °C) and acid magma (750–950 °C) is substantial. The break up into pillow-like units and inclusions results in an increased rate of heat transfer. The time *t* for a thermal wave to penetrate an object radius *R* can be estimated from

$$t = R^2/8K \quad (1)$$

where *K* is the thermal diffusivity. For a typical pillow 1 m diameter and with $K = 3 \times 10^{-7} \text{ m}^2 \text{ s}^{-1}$ (ref. 14) the value of *t* is approximately 29 h. A basic magma that breaks up into metre or decimetre sized units equilibrates thermally with its immediate surroundings in periods of hours or days. Thus the acid magma host would be locally substantially superheated with extreme rapidity.

Magma mixing is believed to lead to vigorous convection in the acid magma. The superheated acid magma at the base of the chamber becomes less dense than the cooler magma above.

The condition for a convective cell to form is when the Rayleigh number (Ra) exceeds 660 (ref. 15) where

$$Ra = \alpha (\Delta T) d^3 g \rho / K \nu$$

In this expression α is the coefficient of thermal expansion ($= 5 \times 10^{-5} \text{ deg}^{-1}$), ν the viscosity, d the length scale of the chamber, K is the thermal diffusivity, ρ is the density of the acid magma, g is the acceleration due to gravity, and ΔT is the difference in temperature of the two magmas. Substituting values of viscosity (10^6 P), d for a magma in a mixing situation (10–1000 m) and temperature difference (10–300 °C) into the above equation yields Rayleigh numbers of 4×10^6 to 10^{13} . The values are orders of magnitude greater than the critical value.

In a simple model of a two-dimensional laminar convection cell¹⁶ the approximate expression for the vertical velocity in the cell is

$$V = (0.25(Ra)^{2/3} K) / d$$

Substituting the range of Rayleigh numbers estimated above, the vertical velocities range from 3.5 m d^{-1} to $3,000 \text{ m d}^{-1}$. These calculations suggest that magma mixing would produce vigorous and rapid stirring of the acid magma in periods of only days or weeks. The calculations are invalid if convection is turbulent, in which case stirring of the two magmas would be even more rapid. Shaw¹⁴ predicted from the work of Lighthouse¹⁵ that at values of Ra greater than 10^4 a magma would undergo fully turbulent convection. Our estimates of Ra indicate that fully turbulent convection is possible in a magma mixing event.

The likelihood of an eruption is greatly increased by magma mixing and convection. The acid magma is mobilised and magma at the base of the chamber is transported to the top. Our calculations indicate this takes place so rapidly that the magma is completely out of equilibrium with its surroundings. If the magma is saturated with volatiles at the base it will become highly supersaturated as it rises and gas pressures will rapidly build up. Increase of temperature in the acid magma reduces the solubility of gases such as water, thus providing a second, independent mechanism for supersaturating the magma. The volume increase due to the intrusion of basic magma is likely to increase the fluid pressure of the acid magma on its chamber walls, and fractures may form providing a pathway for the acid magma to the surface.

At the same time as the acid magma is superheated, the basic magma is quenched and some crystallisation may occur, resulting in exsolution and transfer of volatiles from the basic to the acid magma. Heating of the acid magma reduces its viscosity and ascent of the acid magma through fractures under a constant pressure gradient becomes more rapid and convection more intense as viscosity is lowered. Figure 2 illustrates schematically the model we envisage.

Triggering of acid explosive eruptions by magma has been observed in two geological settings. In regions of rifting such as Iceland magma mixing is the consequence of the fortuitous intersection of basaltic dykes with bodies of rhyolitic magma within acid volcanic centres. Basaltic injections may principally occur during regional rifting episodes such as the volcano-tectonic event in the north-eastern volcanic zone of Iceland from 20 December 1975 to the time of writing¹⁷.

A similar rifting episode occurred in this region from 1874–1875 (ref. 17). The 1875 Askja eruption was preceded by earthquakes and minor activity for a few months before the main event. This rifting episode was accompanied by injection of basaltic dykes as evidenced by the basaltic Sveinagja fissure eruption of 18 February 1875 north of Askja. We suggest that one or more basaltic dykes intercepted the Askja magma chamber (Fig. 2), so that basaltic magma was injected into rhyolitic magma no more than a few weeks or months before the plinian eruption of March 1875.

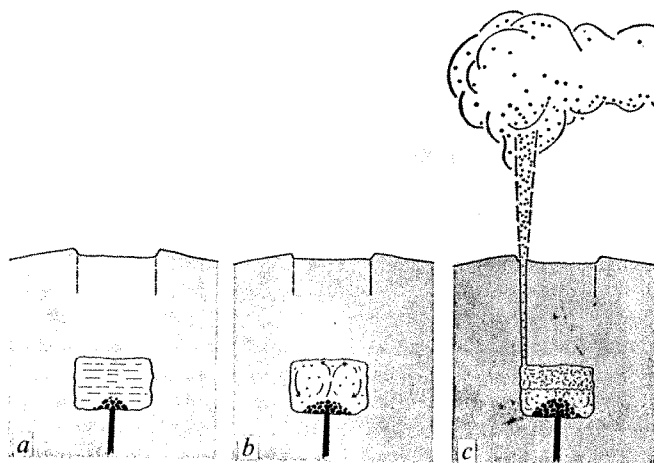


Fig. 2 Schematic model of the mechanism of triggering explosive eruptions by magma mixing. *a*. Basic magma is intruded into the base of acid magma. *b*. Pillows are formed and local superheating of the acid magma induces vigorous convection and vesiculation. *c*. The combination of intrusion of basic melt and vesiculation results in an increase in the fluid pressure of the magma system on the confining walls. Fractures form above the magma and an eruption is triggered.

The convection caused by mixing resulted in entrainment of small pieces of basic magma in the acid magma. These became thoroughly mixed throughout the chamber and so accounted for the simultaneous eruption of rhyolitic tephra with minor amounts of basaltic and mixed tephra from the beginning to the end of the eruption (Fig. 2*b*). At the end of the eruption the level of the magma had fallen to the base of the chamber where the size of basaltic units within the acid magma increases (Fig. 2*c*).

In island arc volcanoes, magma mixing may occur while magma enters from source regions into high level magma chambers. Anderson¹⁸ has provided convincing evidence that some andesite lavas are examples of magmas where both physical and chemical mixing have occurred well before eruption. On Santorini the field evidence is consistent with a small proportion of basic magma being mixed with large volumes of acid magma without substantial chemical interaction shortly before eruption.

In the model (Fig. 2) it is entirely possible that a large part of the basic magma is never erupted. Being considerably denser than their acid host large pieces of basaltic magma will remain at the base. Only inclusions of sufficiently small size will be entrained and mixed throughout the magma. Basic magma may induce convection in acid magma and eruption without being ejected itself. The possibility exists that magma mixing may have a role in eruptions other than those for which there is direct evidence of mixing.

Injection of basic magma into an acid magma chamber results in rapid superheating and induces convection of the acid magma, resulting in entrainment and physical mixing of some of the basic magma. Superheating and decompression of parts of the acid magma due to convection leads to supersaturation of volatile phases and vesiculation. The injection of basic magma and vesiculation of the acid magma markedly increases the pressure exerted by the acid magma on the confining walls. We believe that this pressure increase may often be sufficient to fracture the volcanic edifice and thus trigger an explosive eruption.

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Note added in proof: Another possible consequence of mixing of magmas is water supersaturation due to resorption of calcic plagioclase into the rhyolitic melt, which has been demonstrated experimentally by Yoder¹⁹.

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Magnetostratigraphy of the Cretaceous-Tertiary boundary in the San Juan Basin, New Mexico

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Magnetostratigraphy of a terrestrial sedimentary sequence in the San Juan Basin reveals that the Cretaceous-Tertiary boundary, recognised by the highest stratigraphic occurrence of dinosaurs, is younger than the midpoint of marine magnetic anomaly 29. This boundary is slightly younger than, but very close to, the Cretaceous-Tertiary boundary in the marine sedimentary sequence at Gubbio, Italy, which has recently been proposed as the magnetostratigraphic type section for the Upper Cretaceous and Palaeocene.

FAUNAL succession provides the major basis for recognition of geologic-time units. Boundaries between geologic-time units are placed at points of significant change in the fossil record, such as extinction of major elements of the fauna (here we use 'geologic-time boundary' in the practical sense of boundaries between biostratigraphic units recognised as belonging to different time intervals, but see ref. 1). These boundaries are generally considered to be globally synchronous. Geologic-time units and boundaries are generally defined in marine sequences. These units and boundaries must, then, be correlated with terrestrial sequences on the basis of other criteria. For example, the Cretaceous-Tertiary boundary is defined by extinction of invertebrates (primarily ammonites) in marine sequences but is recognised in terrestrial sequences by the last occurrence of dinosaur fossils. Thus, the global synchronicity of geologic-time boundaries has never been demonstrated. The resolution of this matter has implications for correlation of faunal changes in marine and terrestrial environments, as well as for geochronology itself.

Isotopic dating of horizons within various marine and terrestrial sequences has greatly refined knowledge of age limits and synchronicity of many geologic-time boundaries, but these techniques are not sufficiently precise to resolve the question of global synchronism. To test synchronicity of geologic-time boundaries, a globally synchronous phenomenon must be recorded in both marine and terrestrial sedimentary sequences. Geomagnetic polarity reversals are globally synchronous phenomena² and provide a technique for testing synchronicity of geologic-time boundaries.

Determination of palaeomagnetic polarity stratigraphy in sequences of marine and terrestrial sediments bracketing a particular geologic-time boundary can provide the data necessary to reveal the synchronism or lack of synchronicity of that boundary.

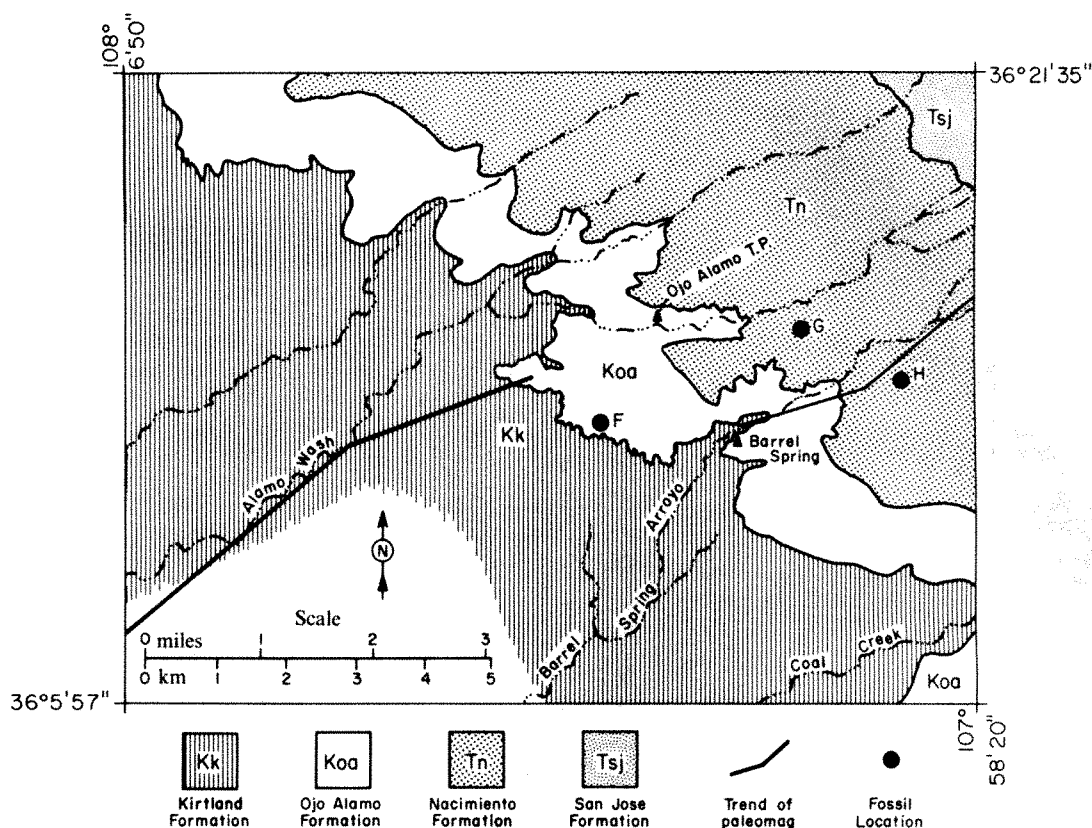
Significant changes occur in both invertebrate and vertebrate faunas at the boundary between the Cretaceous and Tertiary Periods (also Mesozoic and Cainozoic Eras). Through the Deep Sea Drilling Project (DSDP), the earliest foraminiferal zones deposited on oceanic crust of known magnetic anomaly number are providing valuable information on the alignment of invertebrate biostratigraphy with the magnetic anomaly time scale^{3,4}. As a result, considerable progress has been made in referring faunal changes at the Cretaceous-Tertiary boundary in marine sequences to a point in the geomagnetic polarity time scale. These data are complemented by palaeomagnetic polarity determinations on DSDP cores⁵. In addition, Alvarez *et al.*⁶ have proposed a magnetostratigraphic type section for Upper Cretaceous and Palaeocene in Gubbio, Italy. The Cretaceous-Tertiary boundary in this marine sequence occurs near the top of the reversed polarity interval preceding magnetic anomaly number 29.

The San Juan Basin, New Mexico and Colorado, contains a complete Upper Cretaceous into Lower Tertiary sequence of terrestrial sediments. We report here the results of a palaeomagnetic and palaeontologic investigation of the sediments embracing the Cretaceous-Tertiary boundary. These data are an important step toward resolution of the Cretaceous-Tertiary boundary in terrestrial sedimentary sequences, and its comparison with the Italian marine sequence is valuable in evaluating the global synchronism of this important geologic-time boundary.

Stratigraphy and vertebrate palaeontology

The San Juan Basin, located in northwest New Mexico and southwest Colorado, contains one of the most complete Palaeocene terrestrial stratigraphic successions in the world. The Palaeocene sediments are underlain by late Cretaceous and are overlain by early Eocene strata. The terrestrial sedimentary sequence in the San Juan Basin proceeds from shales and interbedded coal layers of the Fruitland Formation, through shales, siltstones and sandstones of the Kirt-

Fig. 1 Trend of the palaeomagnetic section and location of vertebrate fossils. Distribution of geological formations is after Bauer¹². Fossil localities: F, Ojo Alamo local fauna; G and H, *Ectoconus* and *Taeniolabis* zones, respectively, of the Puercan Land Mammal Age.



land Formation, and into conglomeratic sandstone and interbedded shale of the Ojo Alamo Formation. These dinosaur-bearing formations are overlain by varicoloured clays of the Palaeocene Nacimiento Formation and the Eocene San José Formation.

Primary reference faunas for Puercan (early Palaeocene) and Torrejonian (middle Palaeocene) Land Mammal Ages were collected from the Nacimiento Formation⁷. The Tiffanian Land Mammal Age (late Palaeocene) was based on a fauna collected from sediments in Colorado on the north side of the San Juan Basin. These late Palaeocene sediments are usually included in the San José Formation. On the east side of the basin, the San José Formation contains Wasatchian (early Eocene) mammals¹⁸.

The terrestrial sequence in the San Juan Basin is underlain by marine deposits of the Lewis Shale and the Pictured Cliffs Sandstone formations, which correlate with the middle of the Pierre Shale in the interior North American sequence⁸. This indicates that the lower limit of the San Juan Basin terrestrial sequence is equivalent to late Campanian or early Maastrichtian stages of the European Cretaceous succession.

Our palaeomagnetic section (Fig. 1) begins in Hunter Wash and extends to the head of Barrel Spring Arroyo. The lowest faunal level of the Hunter Wash section is the Hunter Wash local fauna⁹ in the Fruitland Formation. This fauna is represented by bone symbols at the 12 m and 33 m levels in the Hunter Wash section (Fig. 2). The Hunter Wash fauna includes fish, turtles, crocodiles, hadrosaur dinosaurs (*Parasaurolophus*), ceratopsian dinosaurs (*Pentaceratops*), carnosaurs dinosaurs, multituberculate mammals (probably five genera), marsupial mammals (probably two genera), and placental mammals (probably two genera)⁹. The highest Cretaceous faunal level, Ojo Alamo local fauna, occurs in the Ojo Alamo Formation at the 256 m level of the Hunter Wash-Alamo Wash section (at F in Fig. 1). The Ojo Alamo fauna includes fish, turtles, crocodiles, hadrosaur dinosaurs (*Kritosaurus*), ceratopsian dinosaurs, carnosaurs dinosaurs and sauropod dinosaurs (*Alamosaurus*)¹⁵. Unidentified mammals have been

found from the Ojo Alamo local fauna, but descriptions have not been published. The Hunter Wash local fauna may be correlated with the Edmontonian or Lancian Land Mammal Ages^{9,10}.

There has been much confusion surrounding the stratigraphic and age limits of the Ojo Alamo Formation^{9,11}. We accept the definition of Bauer¹², which includes the important Ojo Alamo fauna in the Ojo Alamo Formation^{9,13,14}. We also recognise the last occurrence of dinosaurs within the Ojo Alamo Formation. As emphasised by Baltz *et al.*¹⁵, the upper conglomerate with logs of the Ojo Alamo Formation intertongues with the base of the Nacimiento Formation in Barrel Spring Arroyo.

The lowest stratigraphic occurrence of Palaeocene mammals is the *Ectoconus* zone of Sinclair and Granger¹⁴, shown in our Barrel Spring Arroyo section (Fig. 3). The *Ectoconus* zone (G in Figure 1) and the overlying (by about 15 m) *Taeniolabis* zone (H in Fig. 1) are local divisions of the Puercan Land Mammal Age (early Palaeocene). The *Ectoconus* zone is characterised by the abundance of the peripitychid condylarths *Ectoconus*, *Conacodon*, *Hemithlaeus* and *Anisonchus*, and the absence of the large multituberculate *Taeniolabis*. Fossils are not present continuously between the Ojo Alamo faunal level and the *Ectoconus* zone. Thus, the Cretaceous-Tertiary boundary in this terrestrial sequence occurs between these two faunal levels. Accordingly, the boundary is shown as a vertically striped region in Figs 4, 5 and 6.

Magnetostratigraphy

Three oriented block samples were collected at each of 212 palaeomagnetic sites. The nominal stratigraphic separation between sites was 3 m. Details of the collection technique are discussed in ref. 16. Remanence measurements were accomplished with a cryogenic magnetometer (Superconducting Technology, C-102), and alternating field (AF) demagnetisation was performed with a Schonstedt GSD-1 demagnetiser. The average intensity of the natural remanent magnetism (NRM) was $\sim 10^{-6}$ G. Although AF demagnetisation at peak fields of 200–300 Oe was necessary

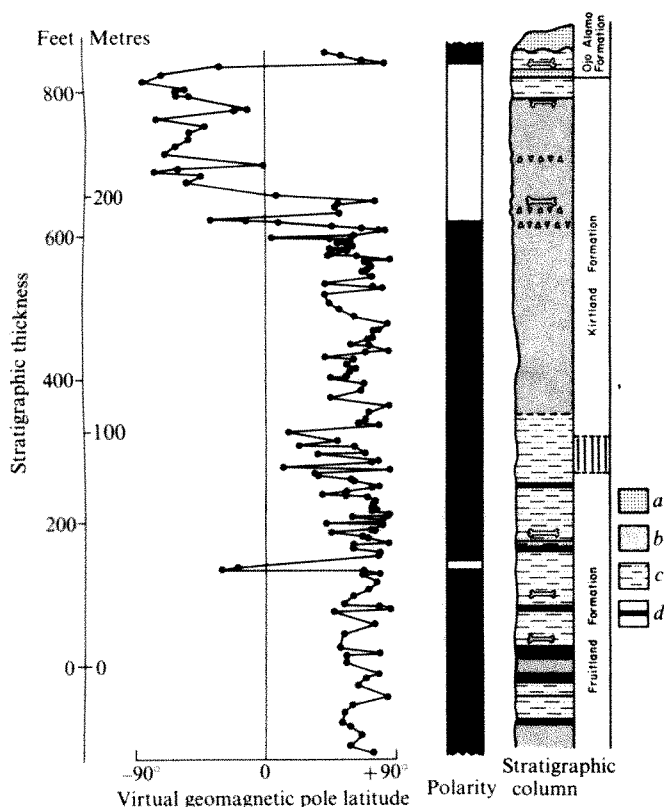


Fig. 2 Hunter Wash-Alamo Wash palaeomagnetic data. Site average virtual geomagnetic pole latitude, resulting polarity column and lithological column are plotted against stratigraphic thickness. Shaded regions in polarity column correspond to normal polarity, unshaded to reversed polarity. Palaeomagnetic samples were AF demagnetised in peak field of 200 Oe. Lines at Fruitland-Kirtland contact indicate the gradational nature of the contact. Bone symbols indicate important vertebrate fossil levels. *a*, Conglomeratic sandstone; *b*, sandstone; *c*, siltstone; *d*, coal layer.

to remove secondary components, unambiguous determination of the polarity of the primary depositional remanence was possible for almost all sites. As there is no accepted procedure for data rejection in palaeomagnetic work on sedimentary rocks of this type, we include all results. It should be recognised, however, that large normal components of viscous or chemical remanence can sometimes obscure or replace a primary reversed NRM. Thus, a certain amount of interpretation must take place in obtaining a polarity log from a set of data. Of particular importance in these interpretations are the motions of the NRM vectors during AF demagnetisation and the degree of clustering of the remanence vectors of the three samples at each site. A more complete discussion of the rock-magnetic properties will be published elsewhere.

Figure 2 shows the site average virtual geomagnetic pole (VGP) latitudes in the Hunter Wash-Alamo Wash section. Positive VGP latitudes indicate normal polarity while negative VGP latitudes indicate reversed polarity. But, incomplete removal of secondary normal components generally prevents VGP latitudes of reversed sites from reaching high southerly latitudes. There are two features of this polarity column which require comment. Although the short reversed polarity interval at the 40 m level is based on only two sites, we are confident this magnetozones is real because of the coherent motion of the NRM vectors during AF demagnetisation. There is some inconsistency of the data at the 190 m level near the bottom of a long reversed polarity zone. We place the bottom of this magnetozones at the lowest occurrence of a well-behaved site

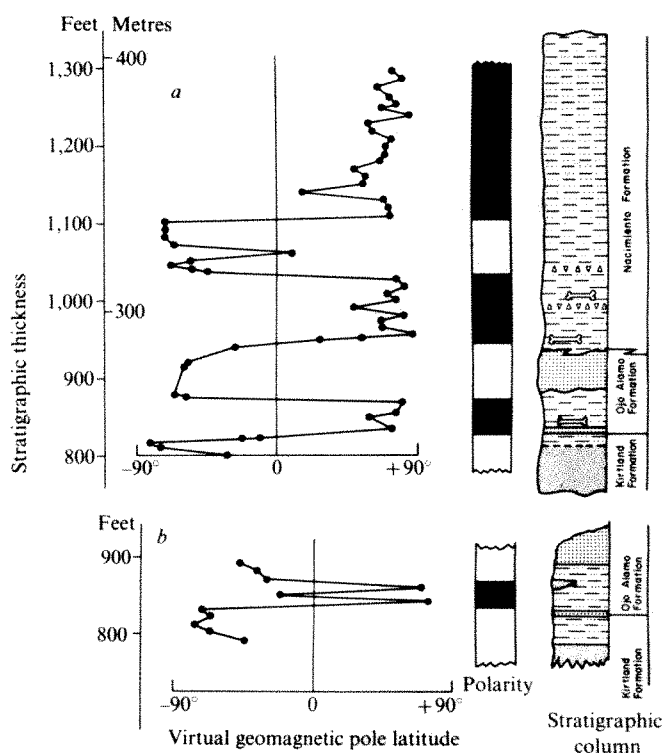
with negative VGP latitude. The stratigraphic interval at 190–200 m is coarse siltstone, and we believe it has been remagnetised by the Earth's present field during weathering of the outcrop.

The palaeomagnetic data for sections in Barnum Brown Amphitheater and Barrel Spring Arroyo are shown in Fig. 3. Again, two features of the polarity interpretation require comment. The site with negative VGP latitude in the middle of the normal polarity magnetozones in Barnum Brown Amphitheater was very weakly magnetised and moved toward positive VGP latitude during AF demagnetisation. Because of these observations and our opinion that magnetozones should not be defined by results at a single site, we favour the polarity column shown rather than a more literal interpretation of the data. The site at the 300 m level in Barrel Spring Arroyo consistently moved toward negative VGP latitude during demagnetisation. Thus, the primary NRM component is of reversed polarity.

The stratigraphic correlation required to construct a composite magnetostratigraphic column through the Cretaceous-Tertiary boundary is shown in Fig. 4. The three sections were lithologically correlated in the field by tracing the lower conglomerate layer and overlying clay layers of the Ojo Alamo Formation between the sections. The total lateral distance between the top of the Hunter Wash-Alamo Wash section and the bottom of the Barrel Spring Arroyo section is only 4 km. The beds are easily traced, and there is little chance of error in this lithological correlation. In addition, the bottom of the normal polarity magnetozones at the 260 m level also correlates between sections and confirms the lithological correlation.

Stratigraphers have generally placed an unconformity at the top of the upper conglomerate layer of the Ojo Alamo Formation and have placed the Cretaceous-Tertiary boundary at that unconformity. The Ojo Alamo and overlying Nacimiento Formation, however, intertongue in Barrel Spring Arroyo. Our section avoided the unconformity at the top of the upper conglomerate by sampling the

Fig. 3 Barnum Brown Amphitheater (*b*) and Barrel Spring Arroyo palaeomagnetic data (*a*). Lithological symbols are as in Fig. 2.



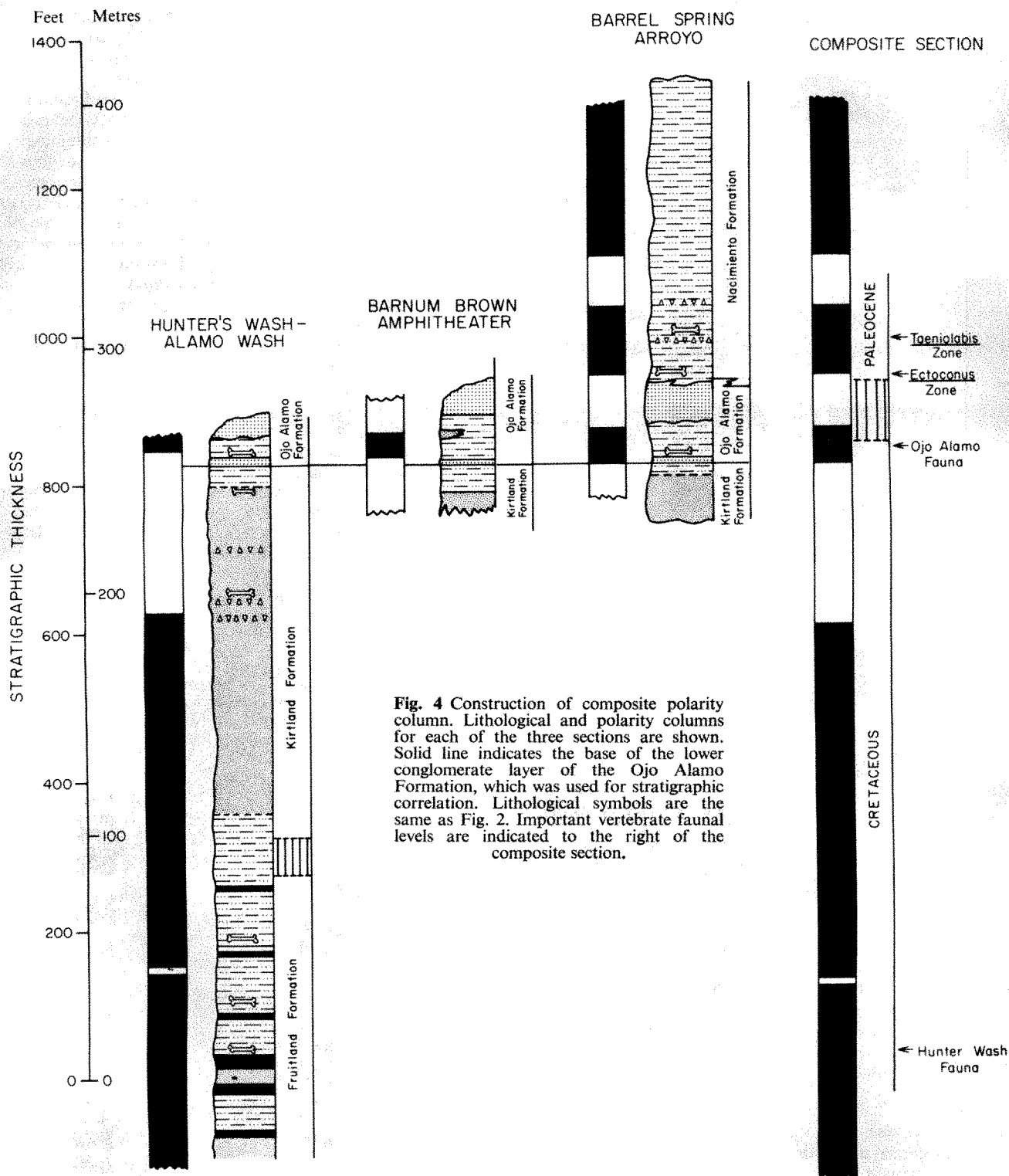


Fig. 4 Construction of composite polarity column. Lithological and polarity columns for each of the three sections are shown. Solid line indicates the base of the lower conglomerate layer of the Ojo Alamo Formation, which was used for stratigraphic correlation. Lithological symbols are the same as Fig. 2. Important vertebrate faunal levels are indicated to the right of the composite section.

Nacimiento Formation in the region of the intertonguing. Tertiary mammalian fossils from the *Ectoconus* zone have been recovered in Barrel Spring Arroyo above the intertonguing while dinosaur fossils occur below the intertonguing. Thus, we have sampled through a continuously exposed stratigraphic section with late Cretaceous and early Palaeocene faunas in superpositional order. We assert that the resulting composite magnetostratigraphic column is a virtually complete section through the Cretaceous-Tertiary boundary. The stratigraphic interval within which the Cretaceous-Tertiary boundary must be placed is shown in Fig. 4.

Another possible unconformity is at the base of the lower

conglomerate layer of the Ojo Alamo Formation. This layer is 2-3 m thick and varies from a homogeneous sandstone to a sandstone with basal conglomerate. There are three lines of evidence which indicate that the lower conglomerate does not represent a significant unconformity. First, the Ojo Alamo faunal zone includes the shales above and below the lower conglomerate. Second, in this region there is a very strong lithological similarity of the shale layers above and below the lower conglomerate. In fact, this similarity is so strong that Brown¹³ and Sinclair and Granger¹⁴ included the shales of the upper Kirtland Formation below the lower conglomerate in their definitions of the Ojo Alamo Formation. And third, as shown below,

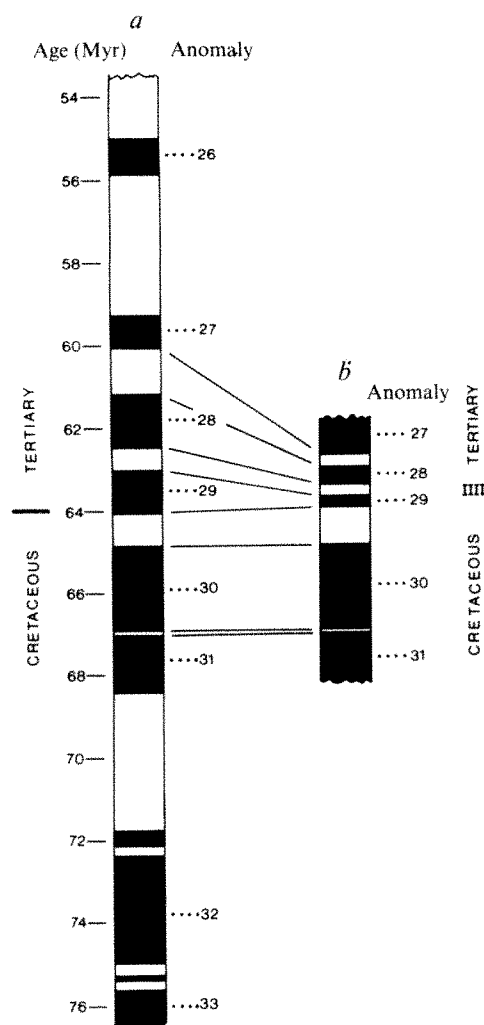


Fig. 5 Correlation of composite polarity column to marine magnetic anomaly time scale. A portion of the Tarling and Mitchell time scale¹⁷ is shown in *a*, while the composite polarity column from the San Juan Basin is shown in *b*. The San Juan Basin polarity column is scaled to make the length of anomaly 30 match the Tarling and Mitchell time scale¹⁷.

there is a very close similarity between the relative thicknesses of the magnetozones in the composite section from the San Juan Basin with the lengths of corresponding polarity intervals in the marine magnetic anomaly time scale. This similarity suggests that no significant unconformity exists at the base of the lower conglomerate of the Ojo Alamo Formation.

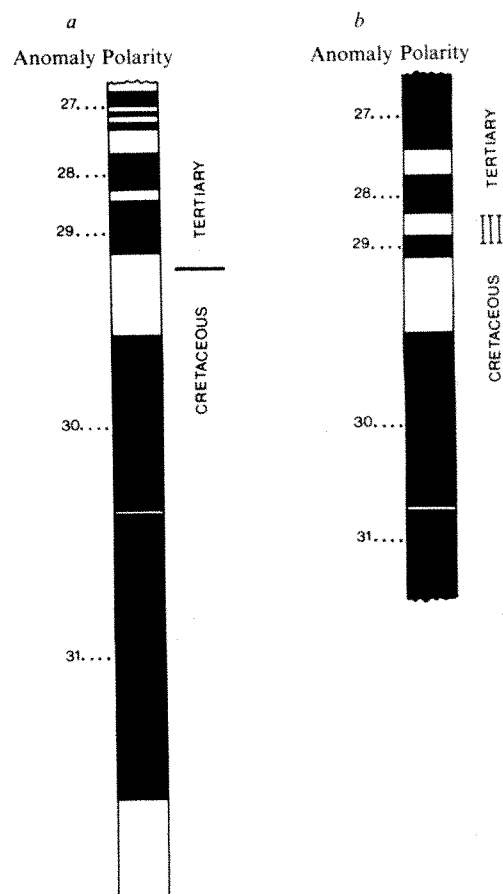
Discussion

Because the lower limit for the age of the Fruitland Formation (as determined from underlying marine formations) is late Campanian to early Maastrichtian, the composite magnetostratigraphic column from the San Juan Basin must be in the late Campanian to Palaeocene interval. Alvarez *et al.*⁸ have shown that late Campanian-early Maastrichtian time corresponds to magnetic anomaly number 33. Thus, none of the magnetozones from the San Juan Basin can be older than anomaly 33. The upper portion of our polarity column is Puercan (early Palaeocene). Evidence from both the magnetic anomaly time scale¹⁷ and magnetostratigraphic studies⁸ indicate that the uppermost magnetozones in our polarity column could not be younger than anomaly 26. Therefore, in attempting to correlate the magnetozones to our composite section with magnetic anomalies, we are constrained to the interval of magnetic anomaly numbers 26-33.

To correlate a magnetostratigraphic section with the anomaly time scale, the section must be of sufficient length and character to allow a unique correlation. A characteristic feature of the marine magnetic anomaly sequence is the very short reversed polarity interval between magnetic anomalies 31 and 30 (Fig. 5). Examination of the polarity sequence in the San Juan Basin reveals the same unusually long normal polarity zone containing one very short reversed polarity zone. In both sequences the long normal polarity interval is followed by more frequent, evenly spaced reversals in the Lower Tertiary. Given the constraints outlined above, the sequence of magnetozones in our composite section contains sufficient character to allow correlation with the magnetic anomaly time scale. The resulting correlation (Fig. 5) indicates that the normal polarity magnetozones in the San Juan Basin section correspond to magnetic anomalies 27 to 31. No other correlation with the magnetic anomaly time scale can be made without assuming very large and rapid changes in sedimentation rate.

Figures 4 and 5 demonstrate the position of the Cretaceous-Tertiary boundary within our stratigraphic sequence. The highest occurrence of in-place dinosaur fossils is toward the top (young side) of anomaly 29 while the lowest Palaeocene mammal fossils are found near the top of the succeeding reversed interval between anomalies 28 and 29. Therefore, the Cretaceous-Tertiary boundary in this North American terrestrial sedimentary sequence occurs in the interval between the middle of anomaly 29 and the beginning of anomaly 28.

Fig. 6 Correlation of magnetozones in *b*, the San Juan Basin and *a*, the marine Scaglia Rossa Limestone of Gubbio, Italy⁶. Only the upper portion of the polarity column from Gubbio, Italy, is shown. The polarity columns were scaled so that the lengths of anomaly 30 are equal.



A correlation of the Gubbio, Italy section with the San Juan Basin section is shown in Fig. 6. This correlation is a graphic illustration of the potential of magnetostratigraphy. Through the intermediate step of correlating these sections to the magnetic anomaly time scale, a very accurate stratigraphic correlation between a marine sequence in Italy and a terrestrial sequence in western North America has been accomplished.

Our data show that the Cretaceous-Tertiary boundary in marine and terrestrial rocks is not synchronous. The Cretaceous-Tertiary boundary, recognised by vertebrate fossils in the San Juan Basin, is younger than the Cretaceous-Tertiary boundary, as recognised by marine invertebrate fossils. The amount of non-synchronicity is small, however. The age difference between the beginnings of anomalies 29 and 28 is of the order of 1.5 Myr (ref. 17). Thus, even the maximum time difference between the Cretaceous-Tertiary boundary at the Gubbio, Italy section and at the San Juan Basin section is probably less than 1.5 Myr. The minimum time difference may be as little as 0.5 Myr. Considering the vast geologic time distance from which we are viewing this event, the relative degree of synchronicity is remarkable.

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New geochronologic and palaeomagnetic data for the hominid-bearing Hadar Formation of Ethiopia

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A 2.6 Myr K/Ar age has been derived for a primary unworked tuff high in the hominid-bearing Hadar Formation (Kada Hadar Member), stratigraphically above all the important fossil finds. A 2.6 Myr fission track age has been derived on zircons from this tuff. New K/Ar results on the Kadada Moumou basalt (Sidi Hakoma Member) suggest a 3.0 Myr age. Preliminary interpretation of a detailed continuous palaeomagnetic section through the formation indicates the existence of persistent normal and reversed sequences. With the radiometric age control this magnetic sequence appears to correlate with the Gauss Epoch. These initial results imply the fossil-rich Hadar Formation spans from somewhat older than 3.1 Myr to somewhat younger than 2.6 Myr.

origin with a rich vertebrate fauna including relatively abundant and excellently preserved hominid material³⁻⁵. In this paper, we present new geochronologic and palaeomagnetic data pertinent to establishing the age of the formation. A geologic column for the Hadar Formation, which represents the area exposed along the Sidi Hakoma and Kada Hadar tributaries north of the Awash River, is shown as section A (Fig. 1). Details of the stratigraphy are presented elsewhere^{1,2}.

Geochronology of Bouroukie Tuffs

Three tuffs have been newly found in the Kada Hadar Member (KHM), which is the uppermost member of the Hadar Formation. We have named these tuffs BKT₁, BKT₂ and BKT₃, from oldest to youngest (Fig. 1), which are well exposed in the Bouroukie tributary to the Kada Hadar drainage. BKT₁ is a 15-cm thick chalky white clay typical of most of the Hadar Tuffs and probably represents completely altered glass ash. BKT₃ is a 75-cm thick buff-coloured glass shard ash with numerous tubular fillings presumed to be root casts. BKT₂ is at present the most

THE well-exposed Plio-Pleistocene Hadar Formation^{1,2} in the central Afar Depression is of lacustrine and lake-margin

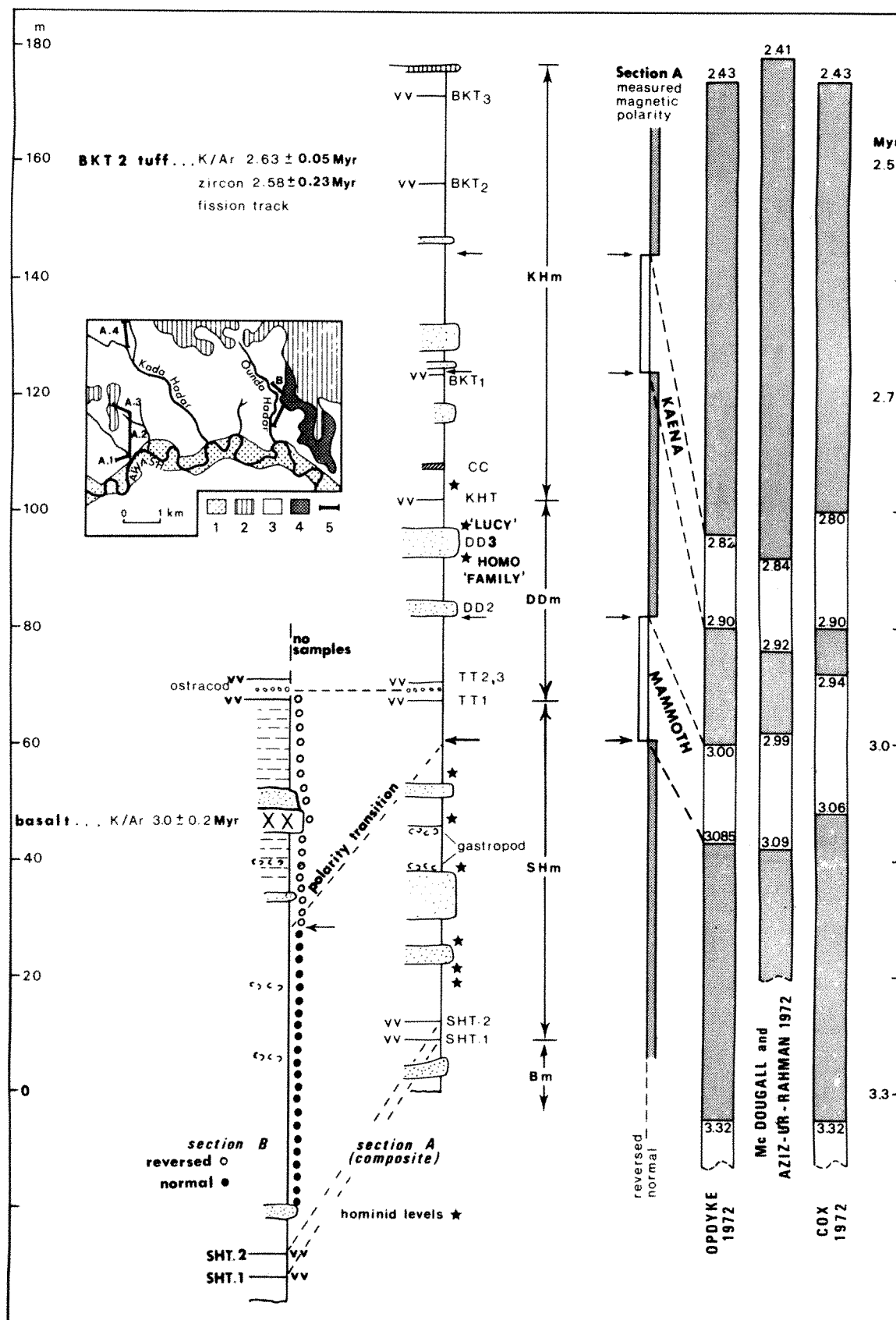


Fig. 1 Palaeomagnetic log of the Hadar Formation. Location of the Gauss Normal Epoch containing the Mammoth and Kaena Reverse Events is shown with reference to three recent versions of the geomagnetic polarity time scale. The important hominid levels are indicated, including the australopithecine skeleton 'Lucy' and the Homo family (site 333). Also shown are all of the tuffs and the basalt and the dates for BKT₂ and the basalt. The members of the Hadar Formation are from the bottom up: Basal Member (BM); Sidi Hakoma Member (SH); Denen Dora Member (DD); and Kada Hadar Member (KH). Legend for the generalised geologic inset map: 1, recent Awash River alluvium and forest; 2, Pleistocene capping gravels; 3, Hadar Formation; 4, Kadada Moumou basalt; and 5, location of palaeomagnetic sections. In palaeomagnetic section B, the open circles schematically represent reversed section and the solid circles represent section of normal polarity.

promising of all the tuffs in the Hadar Formation for geochronology. It consists of two beds separated by 1 m of brown silty clay. The lower bed is a 10–15 cm thick pale yellow crystal-bearing tuff. It is similar to the lower part of the 40-cm thick upper bed on which our geochronology has focused.

The petrology of the upper BKT₂ tuff bed indicates it is of primary pyroclastic origin, uncontaminated by older sediment. The bed has been sharply stratified into two layers by primary aeolian differentiation during the explosive eruption. The upper layer making up two-thirds of this bed is a porous black and white fine lapilli lithic tuff consisting entirely of 1–4 mm grains and granules of rock fragments partially cemented by calcite. The lower layer making up one-third of the bed is a pale yellow crystal and lithic tuff which provided anorthoclase for K/Ar analyses and zircon for the fission-track dating.

The upper lithic tuff layer consists of 65% smaller grains of a brown glass obsidian that is very vesicular (30–50% holes), and 35% larger grains and granules of white (clear) pumice with a spindle-like alignment of glass fibrilles. A gas-rich eruption that became explosive is indicated by these grains, and their coarseness suggests the source was one of the nearer-by Afar acidic volcanoes. Very fine grained basalt (?) forms uncommon inclusions in the obsidian. Both obsidian and pumice have large uncommon phenocrysts of anorthoclase and pyroxene similar to the crystals we will describe from the lower layer. The preservation of fragile broken bubble projections on the obsidian fragments indicates that very little or no rolling transportation occurred. That there was no sedimentary reworking is also indicated by the preservation of the primary aeolian differentiation layering, and the complete lack of any matrix of finer normal density grains for the lower density framework grains of vesicular obsidian and pumice.

The lower one-third layer obtains its pale colour and friability because of the entirely clay-altered 0.5–1 mm grains of collapsed pumice which make up 70% of the layer. Five per cent of the layer is fragments of a magnetite-rich non-vesicular brown obsidian and lesser amounts of a magnetite-rich fine-grained basalt (?). The remaining 25% of the layer is euhedral to subhedral crystals of an exceedingly simple volcanic mineral assemblage: about 24% of a very low-K₂O anorthoclase; 1% green augite rimmed by fibrous amphibole (?); and common magnetite and zircon. All of these four are cogenetic as indicated by the distinctive occurrence of the magnetite as euhedral octahedrons in isolated grains and as very frequent inclusions in each of the other three minerals, and by the occurrence of binary combinations among all the minerals. In addition, the minerals are cogenetic with the obsidian and pumice rock fragments in the upper layer because of their occurrence there as large phenocrysts. It is unlikely the crystals were derived by explosion of a pre-existing rock because all grains are free of any adhering rock matrix. The anorthoclase appears milky under binocular microscope, but clear and unaltered in thin section. It is optically unusual for a potassium feldspar in displaying to various degrees the three major simple feldspar twins (albite, pericline and carlsbad) as well as being untwinned. For example, carlsbad twinned grains occur with one half albite twinned and the other untwinned and optically anorthoclase. Because some grains only display albite twinning, the presence of some plagioclase cannot be ruled out. Staining of the thin section for K₂O, however, developed an equally light measles-like stain on all grains independent of the twinning characteristics, indicating each of the grains is of the same moderately low K₂O content. Additionally suggestive that only one feldspar is present comes from K₂O analyses of seven finely resolved density fractions of the total feldspar. The total range in K₂O was only 1.70–2.00%, with no variation in the four lightest sinks. Thus, no high K₂O feldspar phase exists

in the rock and probably no plagioclase, just the single low-K₂O anorthoclase.

Because of the simple and distinctive mineral assemblage, any extraneous sedimentary components in the lower layer should be apparent. The complete absence of quartz, high-K₂O feldspar, and any variety of rock fragments, and the probable absence of plagioclase, sensitively indicates no admixture of older sedimentary grains has occurred. Together the mineralogy, texture and structure provide abundant evidence that the BKT₂ upper tuff bed is of primary pyroclastic origin with crystals formed at the time of eruption and uncontaminated by older material thereafter.

Four repeat potassium and argon measurements on splits of the same unpicked separate of anorthoclase yield consistent analytically precise ages of 2.65 ± 0.05 Myr (80% radiogenic argon) (Table 1). We report the CWRU laboratory average of 14 measurements of the USGS LP6 Biotite interlaboratory standard because the tracer constant for our bulb-pipette tracer system is presently assigned a value that causes all our age measurements to be 3.5% low in comparison with the average of other laboratories which have run this standard (J. C. Engels, personal communication). Correcting to these other laboratories would raise the 2.6 Myr age to 2.7 Myr. This discrepancy is being evaluated by measuring absolute atmospheric argon standards.

The zircon, which is a common accessory in this sample, has a large range in grain size, from 30 to 120 μ m. All sizes uniformly have a light reddish cast and are euhedral pyramidally terminated prisms. The length to width ratio is variable from 2–10, with stout forms predominant. Eleven grains of the +140 mesh fraction were dated by one of us (C.W.N.) by counting fossil and induced fission tracks (Table 2). The average fission track age of the eleven grains is 2.58 Myr with a standard deviation of ± 0.23 Myr.

Because all of the Hadar Formation fauna recovered so far is stratigraphically below BKT₂, these concordant K/Ar and zircon fission track ages, and the unlikelihood that the tuff is contaminated, indicate that the fauna is older than 2.6 Myr.

Geochronology of Kadada Moumou basalt

The stratigraphic position of the Kadada Moumou basalt flow at the eastern edge of the Hadar site is now established within the upper part of the Sidi Hakoma Member. The Kadada Moumou subplateau is upheld by the 1–4 m thick resistant basalt flow which is capped unconformably by mid-Pleistocene (?) gravels and sands with Acheulean artifacts⁶. Near the northwest end of the basalt some grey mudstone above the basalt may represent the only Hadar Formation sediment not stripped by erosion from above the basalt. The basalt is 70 m above the Sidi Hakoma Tuff (SHT) which forms the base of the Sidi Hakoma Member. The basalt was erupted on a gentle erosional surface of subdued topography with local relief up to about 10 m. The northwest end of exposure of the basalt probably represents a slightly eroded flow front. A cross-section of a large 10 m deep palaeo-channel or palaeo-embayment is exposed along this flow front. We have established that the channel is filled with Hadar-age sand beds which post-date the basalt and which can be related to the overlying Hadar sequence. The channel fill sand beds have original dips parallel to the approximate 20° slope leading downward from the basalt. The initial sand bed in the channel contains numerous boulders and cobbles of the vesicular basalt and thus post-dates the basalt. These channel-fill sands are of undoubted Hadar Formation age because they contain abundant Hadar fauna. One basalt boulder in the basal sand of the channel was found within the metre-wide carapace of a fossil turtle (discovered by B. T. Gray). Conformably above the channel fill is a distinctive extensive sand horizon with abundant root casts. It can be traced from the basalt front directly

into the main Hadar sequence, where it lies 20 m below the triple tuff marker horizon that is the top of the Sidi Hakoma Member (SHM) (Fig. 1). This marker has been walked out to the main fossil bearing localities in the Kada Hadar drainage. Thus, the basalt erupted on an erosional surface that is probably minor within the Sidi Hakoma Member at the eastern edge of the Hadar site. In the Kadada Moumou area, it is located 70 m above the base and 20 m below the top of the member. Further west in the main part of the Hadar site the Sidi Hakoma Member has a total thickness of only 60 m (Section A) compared to the 90 m in the east. A more detailed correlation of the

The average of six K/Ar analyses of three geographic samples of the shiny black variety is 3.0 Myr with an average deviation of ± 0.06 Myr (Table 1), including our two previously reported results¹. In contrast, one sample of the more altered sugary-textured variety gives an age of 2.65 Myr (two analyses) and another sample, which is a little more altered, an age of 2.50 Myr. Each individual analysis of the Kadada Moumou basalt has an estimated uncertainty of ± 0.2 Myr, because the extracted argon was only 10–20% radiogenic.

A key factor in interpreting the K/Ar discrepancy between the two varieties is secondary mineralisation, which

Table 1 K/Ar Data

Sample	Weight (g)	⁴⁰ Ar* per g	⁴⁰ Ar*/ ⁴⁰ Ar (×100)	%K ₂ O†	K–Ar age (Myr)‡
<i>a</i> , BKT ₂ -tuff					
38–75–1 Anorthoclase					
35/80 mesh					
no. 1§	5.4	0.0774	84.8	2.00±0.01(2)	2.62±0.08
no. 2	3.8	0.0740	76.8	1.96±0.004(2)	2.55±0.08
no. 3	4.4	0.0784	67.1	1.96±0.004(2)	2.70±0.08
no. 4	4.2	0.0766	79.8	1.96±0.004(2)	2.64±0.08
					2.63±0.04(4)†
<i>b</i> , Kadada Moumou Basalt					
(1) Massive black phase					
KM					
1–73 ₁ ¶	10.0	0.0291	20.6	0.657±0.008(11)	3.00±0.20
1–73 ₂ ¶	10.0	0.0291	15.9	0.657±0.008(11)	3.00±0.20
2–74 ₁ ¶	10.0	0.0318	15.2	0.755±0.008(9)	2.95±0.20
2–74 ₂ ¶	10.0	0.0326	13.3	0.755±0.008(9)	2.90±0.20
4–74 ₁	10.0	0.0318	13.9	0.68±0.01(5)	3.15±0.25
4–74 ₂	10.0	0.0308	13.0	0.68±0.01(5)	3.05±0.25
					3.00±0.05(6)†
(2) Sugary grey phase					
KM					
1–74	10.0	0.0326	13.3	0.727±0.008(5)	2.50±0.25
3–74 ₂	10.0	0.0295	25.8	0.747±0.005(5)	2.65±0.20
3–74 ₂ **	10.0	0.0298	14.4	0.747±0.005(5)	2.70±0.25
LP 6 Interlaboratory biotite standard	0.24†	18.44±0.36 (19)†	95 (19)	10.14±0.08(18)	

* Radiogenic argon, 10^{-10} mol g⁻¹.

† Average and average deviation, no. of measurements in parentheses.

‡ ⁴⁰K/⁴⁰Ar total = 1.19×10^{-4} ; $\lambda_b = 4.72 \times 10^{-10}$ yr⁻¹; $\lambda_e = 5.85 \times 10^{-11}$ yr⁻¹; age uncertainty for individual samples calculated using 0.3% uncertainty in isotopic composition of sample and atmospheric argon, a 1% uncertainty in argon spike, 1% uncertainty in K₂O, and 1% uncertainty in sample homogeneity.

§ Treated, 4% cold HF 1 minute, resieved to +100 mesh.

|| Low temperature fusion within a previously fused and degassed basalt.

¶ Analysis previously reported, ref. 1.

** Sample not pre-baked before extraction.

basalt position within this thinner section to the west is suggested in the discussion on magnetostratigraphy (Fig. 1).

Several K/Ar results on the basalt fall into two groups which correlate with the two subtle varieties of the flow selected from fresh cores of large talus blocks. These varieties probably represent a different vertical position in the flow not yet observed. One variety is a relatively unaltered dark black rock breaking with a smooth surface compared to a dark grey more granular or sugary-surfaced variety which thin section study indicates is relatively more altered. Both types consist of fine-grained plagioclase, augite, magnetite and minor olivine in a cryptocrystalline glassy matrix. The dark grey granular variety is coarser grained (0.2–0.4 mm compared with 0.15 mm), and contains much less of the cryptocrystalline matrix (10% compared with 35%) than the smooth black variety. The smooth black variety largely obtains its character from this abundant cryptocrystalline matrix, charged with magnetite dust.

occurs in two ways: (1) a filling of cavities, which occurs in both varieties of basalt and is interpreted not to have affected the age measurement; and (2) a yellowish clay alteration of the glassy matrix which affects only the grey variety and could well have caused argon loss. As regards the secondary filling, both varieties have a diktytaxitic texture (angular interstitial cavities) with the smaller cavities filled with a yellowish clay (?) and larger cavities lined with the clay concentric about a coarse calcite filling. This secondary filling is the same observed in the abundant vesicles at the top of the flow which may be empty, lined with clay, or lined with clay around calcite. A complete gradation can be observed in the same outcrop between samples with only fine angular interstitial cavities and samples with only normal rounded vesicles. As regards the secondary alteration, which affects only the grey variety of basalt, about 10% of the cryptocrystalline glassy matrix areas of KM-3-74, and about 50% of the matrix areas in

KM-1-74, have interior regions which have altered to a yellowish orange clay (?). The altered zone cuts across and includes relict magnetite grains. Because of the relatively small proportion of glassy matrix in the grey variety (10%), a significant portion of the rock's K_2O can be expected to have been residually concentrated in the glassy phase; if the alteration occurred much later than the eruption, it would have resulted in argon loss and reduced the age measurement. Because the shiny black variety has no alteration of its large amount of glassy matrix (35%), and because there is an indication that the smaller amount of clay-like material in this rock is not an alteration but a secondary filling, we interpret its K/Ar system to be unaltered. To be conservative, its 3.0 Myr age should be regarded as a minimum age because a coexisting variety of the flow, which has endured a similar history, has demonstrably lost argon compared to it.

Magneto-stratigraphy

Studies at East Turkana (formerly Lake Rudolf)⁷ and at Omo⁸ have demonstrated the usefulness of magneto-stratigraphy as a geochronological tool in the terrestrial Plio-Pleistocene sediments of East Africa. One of us (T.J.S.)

been deposited during a time of reversed polarity, with the irregularity resulting from partial remagnetisation during exposure to the present normal polarity. Until we are able to establish the precision of the boundaries by more widespread sampling, the uncertainty in the stratigraphic position of the polarity transitions is assumed to be within 10 m. Figure 1 shows the location of these polarity transitions in sections A and B.

Taken together with the absolute age control, provided primarily by the 2.6-Myr BKT₂ tuff and secondarily by the 3.0-Myr basalt age, the dominantly normal palaeomagnetic stratigraphy of the Hadar Formation is interpreted to be of Gauss Epoch age. The two reversed sequences would represent the well-documented Mammoth and Kaena Events which took place about 3.0–3.1 Myr ago and 2.8–2.9 Myr ago, respectively, as determined by numerous geochronologic studies elsewhere in the world^{9–11}. This correlation with the world-wide reversal scale is shown in Fig. 1.

The palaeomagnetic results of the 30 samples collected the previous field season from three parts of the Hadar Formation¹ are perfectly in accord with the new detailed stratigraphy presented here. One problem arises in correlating partial section B of the Ounda Hadar area with

Table 2 Fission track dating results for BKT₂ (tuff)

Sample	No. of zircons	Spontaneous tracks counted	Ps (cm ⁻²)	Induced tracks counted	Pi (cm ⁻²)	Neutron flux (n cm ⁻²)	Uranium content p.p.m.	Age (Myr)*
38-75-1 +140 mesh	11	140	2.68×10^5	1,495	5.715×10^6	9×10^{14}	190	2.58 ± 0.23

* Mean $\pm$ 1 s.d.

has started a detailed study of the palaeomagnetism of the Hadar Formation. The preliminary results suggest that a magnetic stratigraphy is retained which can be correlated with the palaeomagnetic polarity reversal time scale. So far only one complete section of the formation has been taken, sampling approximately every half metre. This section (section A, Fig. 1) is in the Sidi Hakoma and Kada Hadar drainage, where most of the fossil localities of the Hadar site occur. In addition, a partial section (section B, Fig. 1) with irregularly spaced samples from 1–4 m apart has also been measured in the Ounda Hadar drainage, including the basalt flow. A total of 400 samples were collected.

All the samples were subjected to progressive alternating field (A.F.) demagnetisation in order to determine the direction and stability of their remnant magnetism. The details of the magnetic behaviour of individual samples will be presented after more sections are completed and only a summary is reported here. The major result is that after 200 Oe of A.F. demagnetisation, the remnant magnetism of individual samples falls into three sequences of very uniform normal polarity, and two sequences of predominantly reversed samples with intermixed samples of random polarity. Every tenth sample of section A (about every 5 m) was subjected to further progressive demagnetisation up to 1,000 Oe. All of these further demagnetised samples in the three normal zones maintained their persistent normal polarity with directions close to north. The randomly interspersed intermediate samples from the dominantly reversed sequences which were defined at 200 Oe of demagnetisation, generally revealed a reversed or more nearly reversed remnant direction on further demagnetisation. The reversed samples in these sequences maintained their reversed polarity. Thus, the higher field demagnetisation enhanced the contrast evident between the two kinds of sequences. We interpret the two sequences with dominantly reversed remnant magnetisation to have

Section A, because 40 m of reversed sediment occur below the ostracod marker bed in Section B and only 10 m occur below this marker in Section A. The thickness of the Sidi Hakoma Member in Section B is 90 m compared to only 60 m in Section A. The present magneto-stratigraphy suggests that most of this east to west variation in thickness of the Sidi Hakoma Member can be ascribed to the very upper part of the member. To what extent this thickness variation is due to varying rates of deposition or to unconformities is not known.

If interpretation of the age framework of the Hadar Formation continues to be substantiated, then more detailed palaeomagnetic studies may allow for additional precision in the stratigraphy, palaeogeography and faunal evolution at the Hadar site. At present, the geochronology and palaeomagnetism suggest for the Hadar Formation and its rich fauna a Gauss Epoch age that spanned from somewhat more than 3.1 Myr ago to somewhat less than 2.6 Myr ago.

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letters to nature

The rings of Uranus

At least five rings encircle the planet Uranus—as indicated by five brief occultations of the star SAO 158687 that occurred both before and after its occultation by Uranus on 10 March 1977. We observed these events with our three-channel occultation photometer¹, attached to the 91-cm telescope aboard the Kuiper Airborne Observatory (KAO). Both Uranus and the star were contained within a focal plane aperture whose diameter, projected onto the sky, was 46 arc s. A beamsplitter and focal plane television system allowed us to monitor the position of the image in this aperture simultaneously with the photometric measurements. The wavelengths of the three photometric channels used were chosen to yield favourable ratios of starlight to Uranus light² and are given in Table 1. For each channel a cooled photomultiplier (RCA C 31034), connected to a photon-counting system, was used as a detector. The data were recorded as a continuous series of 10-ms integrations of photon counts, and a strip chart recorder displayed the signals from our channels 2 and 3 so that we could monitor the progress of the occultation in real time.

Table 1 Centre wavelengths of filters

Channel no.	Centre wavelength (Å)	Passband (Å)	Star counts* Star+background counts
1	6190	80	0.15
2	7280	200	0.32
3	8520	210	0.42

*These ratios were obtained from the observed signal levels.

The final predictions^{3,4} for the occultation indicated that the northern limit of the occultation would pass south of northern Australia, but its precise location was uncertain. Accordingly, the course of the airborne observatory was planned to be as far south as possible. At about 20 h 00 min UT, the KAO assumed a course that allowed tracking on Uranus, and continuous data recording began at 20 h 05 min 40 s UT. A sharp decrease in the recorded light intensity occurred at 20 h 11 min 43 s UT and several seconds elapsed before the signal returned to its original level. This event was followed by several others of shorter duration within the next few minutes. The cause of these events was initially thought to be momentary failures of the telescope tracking system, but careful examination of the Uranus image on the television monitor showed the tracking to be steady. Another cause considered was thin cloud, but at our altitude of 41,000 feet this was unlikely. The infrared water vapour monitor on board the KAO confirmed that no clouds were present. We concluded that the drops in intensity were definitely light lost from the star, since the dips had the proper ratios at different wavelengths (see Table 1). For this case the only plausible cause of these brief intensity dips would be occultations by small bodies, probably associated with Uranus.

About 30 min later, Uranus occulted the star. The occultation lasted about 25 min and had a midtime of 21 h 06 min UT, in good agreement with the predictions based on the

position of the star and Uranus determined by Franz and Wasserman⁵. We then continued our observations in expectation of recording more brief occultations. Five such events were recorded before the data recording was stopped at 22 h 17 min 40 s UT, due to the high level of morning twilight. Similar brief occultations of SAO 158687 when Uranus was near it were also observed by Millis, Birch and Trout at Perth^{3,6}; by Bhattacharyya and Kuppuswamy at Kavalur^{5,6} and by Churms⁶ at Cape Town.

Table 2 contains the midtimes and approximate durations of the brief occultations that we observed. The approximate location of the Airborne Observatory was lat. 50°20'S, long. 82°11'E for the pre-immersion events and lat. 50°21'S, long. 101°19'E for the post-emersion events. In Fig. 1 we have plotted our light-curves (channel 2) for both sets of events after averaging the data over intervals of 0.5 s. The intensity parameter $\phi(t)$ equals 1.0 for the unocculted intensity of SAO 158687 and 0.0 when the star is totally occulted. We have not attempted to remove from these data possible long-term drifts in sky background light and instrumental gain, which could amount to a few per cent. The slow rise in signal level starting at 21 h 52 min UT is caused by morning twilight.

We assumed that the bodies causing the occultations lie in the satellite orbit plane of Uranus and computed their distance from the centre of Uranus. The angle between the line of sight and the normal to the satellite orbit plane was 36.6°. In this computation, the radius of Uranus was set equal to 25,900 km (ref. 8). Then the chord length observed when Uranus occulted the star⁵ was used to fix the position of Uranus relative to the star. The motion of the KAO was not taken into account in this calculation, however, thus introducing systematic errors of several hundred km, which can ultimately be eliminated. But, since the groups of events occurred at approximately equal times before and after the Uranus occultation itself, the errors in the relative distances between the immersion and emersion events should be less than 100 km.

Table 2 Short occultation events

Pre-immersion events		Post-emersion events	
Midtime (UT) h min s	Approximate duration (s)	Midtime (UT) h min s	Approximate duration (s)
20:11:46	7	21:45:52	1
20:16:03	1	21:47:06	1
20:16:58	1	21:49:45	1
20:19:34	1	21:50:39	1
20:20:55	1	21:54:06	3

The striking impression from Fig. 1 is that the occultations in both groups of events occur at corresponding distances from Uranus. Moreover, the amplitudes and durations of the corresponding events are similar. We feel this is strong evidence that the occultations were caused by five narrow rings that encircle the planet, rather than random occultations caused by small satellites within a broad belt as we and the Lowell group had originally suggested^{3,9}. We have tentatively labelled the rings with the letters α – ϵ , beginning with the ring closest to Uranus.

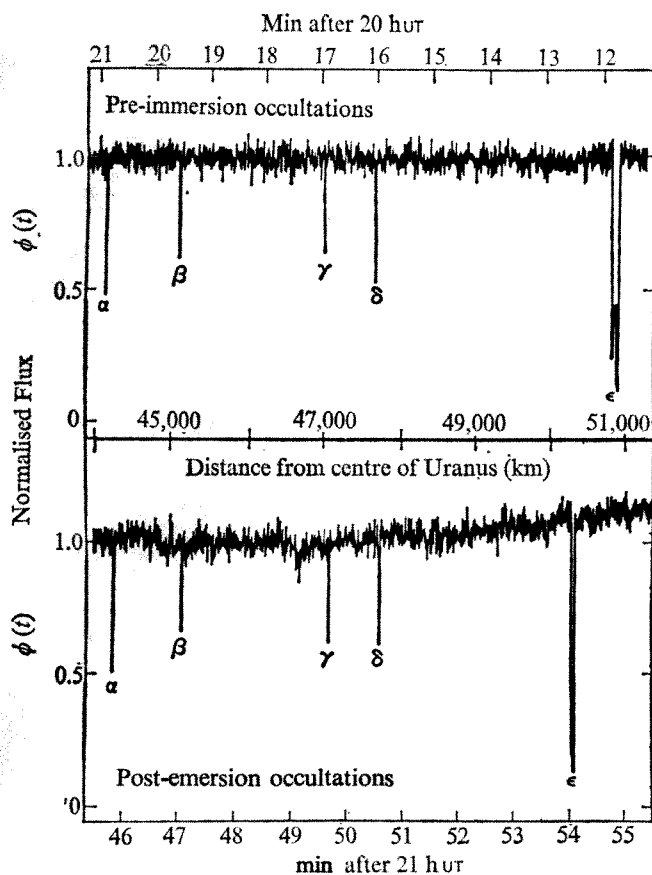


Fig. 1 Light curves (channel 2) showing the five brief occultations observed both before and after the occultation of SAO 158687 by Uranus on 10 March 1977. The intensity scale $\phi(t)$, has been normalised to the unocculted intensity of SAO 158687, and on this scale the amount of starlight occulted ranges from about 0.4 (β and γ events) to about 0.9 (ϵ events) of the total. An approximate distance scale has been computed under the assumption that the bodies causing the occultations lie in the orbit plane of the Uranus satellites. The equal number and symmetry of the occultations suggest that four narrow rings (~ 10 km wide) and a broader ring of variable thickness (ring ϵ , 50–100 km) encircle Uranus. Beginning at 21 h 52 min UT, the start of twilight caused a slow rise in the signal level.

Since the KAO data was the only set reported to contain both the pre-immersion and post-emersion occultation events, we were in a unique position to infer the presence of narrow rings. Subsequent calculations by Marsden⁷ showed that the times of the events observed at Perth and Cape Town led to distances from Uranus, within its satellite orbit plane, that closely agreed with those obtained from our data. Hence the interpretation that these events were caused by narrow rings encircling Uranus has already survived a stringent observational test. (We note a ~ 800 km discrepancy between Marsden's distance scale and our own that arises from different assumptions for our respective calculations. But, the distances of corresponding pre-immersion and post-emersion events are nearly equal for either calculation.)

Our light curves also contain other brief occultations, significantly shallower than the ring α - ϵ events, and are probably caused by additional material within the ring system of Uranus. One example is the decrease in signal that occurs shortly before the ring γ occultation on emersion (see Fig. 1), and there is some indication in our data of similar occultations that correspond to the latest two events reported by Millis, Wasserman and Birch⁷. Our figure does not include this time interval. As yet we have not searched our data for long, shallow occultation features that would correspond to broad rings of low optical depth.

Ring ϵ seems markedly different from the other four in

several respects. It causes much deeper occultations of the star that last longer, but are of unequal duration (Table 2) for the two sections of ring ϵ probed by our observations. Also the times of the occultations by ring ϵ do not agree with its being circular and in the plane of the satellite orbits.

Possibly ring ϵ is elliptical, or has an orbit plane inclined to that of the satellites; it may be that what we have identified as a single ring is actually two incomplete rings. From our data alone, we cannot confirm or rule out any of these explanations, and as yet cannot suggest a more sophisticated model to explain the times and durations of the occultations caused by this ring.

To estimate the width of the rings, we note that the Uranus shadow velocity was 12 km s^{-1} and use the occultation durations given in Table 2. These values yield a width of ~ 12 km, projected onto the plane perpendicular to our line of sight, for rings $\alpha, \beta, \gamma, \delta$; ~ 85 km for the immersion section of ring ϵ ; and ~ 35 km for the emersion section of ring ϵ . We emphasise that these widths are only approximate, since we did not include (1) the convolution broadening of the observed occultation durations caused by the projected diameter (~ 6 km; see ref. 10) of the star at Uranus; (2) diffraction effects (Fresnel scale, $\sqrt{\lambda R} \sim 1.5$ km); (3) the projection angle between the apparent stellar velocity and the orientation of the ring; and (4) effects caused by the orbital motion of the rings, with a velocity of 11 km s^{-1} , that would be important if the optical depth of the rings is not uniform on scales comparable to, and larger than, the projected stellar diameter.

Since none of the rings caused a total occultation of the star, we think they are composed of numerous bodies, with sizes of < 6 km (the projected diameter of the star), rather than larger objects. There are no obvious differences in the structure of the occultations at the different wavelengths that we observed. Such differences would be indicative of diffraction effects and/or wavelength dependent extinction and perhaps provide an important clue to the nature of the occulting material. We plan to pursue this analysis further.

Valuable observations of future occultations by the rings can be obtained for stars significantly fainter than SAO 158687 ($m_v \sim +9.5$; see ref. 10); the precise limiting magnitude will depend on the spectral type of the star and the aperture of the available telescope². Since these occultations need not involve an occultation by Uranus itself, we suggest that present lists of appulses involving Uranus be reviewed in search of those that could result in occultations by the rings. Also, future occultation predictions for Jupiter, Uranus, and Neptune should include information about the apparent path of the star relative to the satellite orbit planes of these planets. The possibility that Jupiter has rings comprised of 'rocky' bodies cannot at present be excluded¹¹. Saturn's rings may contain narrow components, of much higher optical depth than the average, akin to the rings of Uranus. An opportunity to check this possibility, but with coarser spatial resolution than the present measurements for Uranus, occurs in October 1977 and January 1978 when the satellite Iapetus is eclipsed by Saturn and its rings¹².

Possibly the Uranus ring system can be detected through direct photography. If the rings have the same albedo as the cloud tops of Uranus, their combined integrated intensity should be $\sim 0.7\%$ of that of the Uranus disk. This ratio should be substantially more favourable for wavelengths within the methane absorption bands².

The distances between the various rings pose an interesting theoretical problem that can possibly be explained by resonances with orbits of the known satellites and will perhaps require the inference of another satellite. All five rings are well within the Roche limit, which is about 70,000 km for Uranus.

The fundamental question of why rings were unique to

Saturn has been posed many times in the past¹¹, and the particular case of why Uranus was thought to have no rings has been addressed also¹². We now ask, why are the rings of Uranus so narrow—in contrast to the broad rings that surround Saturn? The possibility of 'very small bodies, similar to the asteroids' being found between the orbit of the inner-most satellite Miranda and Uranus itself has been mentioned by Alfvén and Arrhenius¹³; the narrow rings may well be a manifestation of the 'jet streams', discussed at length by the same authors¹³. The theoretical basis for jet streams has not won general acceptance¹⁶, however, and we expect that alternative theories for the narrow rings will become available soon.

We thank the staff of the Kuiper Airborne Observatory, headed by Carl Gillespie, for assistance and acknowledge our close association with R. L. Millis and L. H. Wasserman of Lowell Observatory in planning our respective observations and in comparing results. Also, we are grateful for discussions with B. Marsden, J. Veverka, D. Hunten, G. E. Taylor, W. B. Hubbard and Carl Sagan that generally contributed to the success of this project. This work was supported by NASA and E.D. is supported in part by an Allen Seymour Olmsted Fellowship.

Note added in proof: W. B. Hubbard has reported that three ring occultations were observed by B. Zellner at Perth Observatory.¹⁷

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Detection of rings around Uranus

AN occultation of the ninth-magnitude star SAO 158687 by Uranus on 10 March 1977 was predicted by Gordon Taylor¹ in 1973. Although the original prediction indicated that the occultation would be widely visible from land masses surrounding the Indian Ocean, subsequent work by Franz and Wasserman (at Lowell Observatory) showed that ground-based observations would be possible only from southern Africa and possibly from southwestern Australia². In view of the wealth of new information about Uranus which could be derived from photometric observations of this event³, we decided to attempt observations using the 61-cm Planetary Patrol telescope at Perth Observatory near Bickley, Western Australia. In fact, an occultation of the star by Uranus itself did not occur at Perth. Partial occultations of SAO 158687 by a series of rings around the planet were observed and are described here.

Measurements were made using a conventional single-channel photometer equipped with an RCA C31034B photomultiplier. The observations were made through an

interference filter centred at 8524 Å with a width of 212 Å (FWHM). Since this filter coincides with a strong methane band in the spectrum of Uranus, the signal from SAO 158687 relative to that from Uranus was greatly enhanced³. An identical filter was used by Elliot *et al.* in one channel of their multi-colour observations of this occultation⁴. The signal from the photomultiplier was amplified by a Kiethley Model 301 operational amplifier and displayed on a strip-chart recorder. The response time of this system for a full-scale deflection is less than 0.5 s. In addition, the signal from the amplifier was fed to a voltage-to-frequency converter whose output was recorded on magnetic tape. This feature provides for digital output of the data at high time resolution, but its recording capacity is limited to 45 min.

During the observations, Uranus was centred in a 28-arc s diameter aperture. Measurements started at 20 h 00 min 00 s UT and continued well into morning twilight with brief interruptions at 5-min intervals for recentering the planet. It is clear from these observations that an occultation of SAO 158687 by Uranus itself did not occur at Perth. But, between 20 h 10 min and 20 h 24 min—well before the predicted time of the occultation of the star by Uranus and before the digital recording system had been turned on—five brief partial occultations of SAO 158687 were detected with the chart recorder. The midtimes, durations, and depths (total occultation=1.0) of these events are listed in Table 1. We estimate a maximum uncertainty in the listed midtimes of ± 0.2 s.

Table 1 Occultations of SAO 158687

Event	UT h min s	Duration (s)	Depth
1	20 10 33.2	8.2	0.94–0.52
2	20 15 58.7	0.7	0.42
3	20 18 44.1	1.5	0.38
4	20 23 19.0	1.0	0.21
5	20 23 42.4	0.9	0.29

Several similar partial occultations were observed by Elliot *et al.*, observing aboard the Kuiper Airborne Observatory about 2,000 km south-southwest of Perth, by Churms at Capetown and by Bhattacharyya and Kuppaswamy at Kavalur^{5,6}. A cursory comparison of the aircraft data and the Perth observations a few hours after the occultations led to a preliminary conclusion by both groups of investigators that several individual members of a satellite belt around Uranus had been detected⁵. The Cornell investigators subsequently discovered, however, that the occultations which they observed prior to the Uranus occultation were very nearly mirror images in time of those which they observed following the Uranus occultation. It was then concluded that the occulting bodies were five narrow circular rings in the equatorial plane of Uranus^{4,6}. These rings have been designated α , β , γ , δ , and ϵ , starting with the innermost ring. The 61-cm Perth observations support this interpretation. If we predict when occultations by these five rings would have occurred at Perth, we find that event number 1 in Table 1 corresponds to the ϵ ring, event 2 corresponds to the γ ring, and event 3 corresponds to the β ring. We did not observe the occultations by rings α and δ at Perth because at their predicted times of occurrence, the observations had been interrupted for about 10 s to recentre the planet. Events 4 and 5, although shallower than the other occultations, are well above the noise level of our observations. It is not yet clear whether these events are present in the observations of other observers, so the nature of the occulting objects is uncertain in these two cases. If these objects are

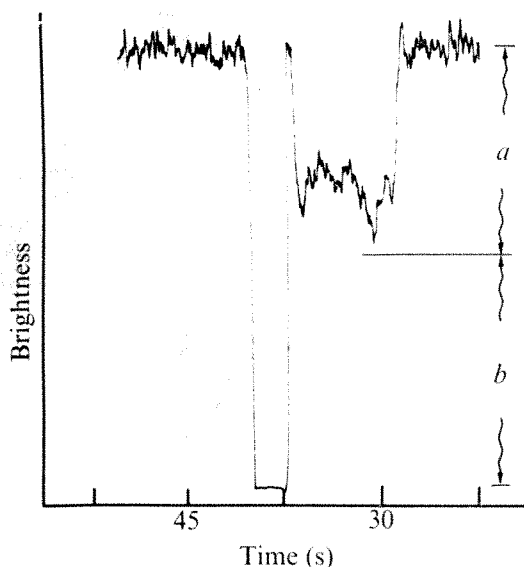
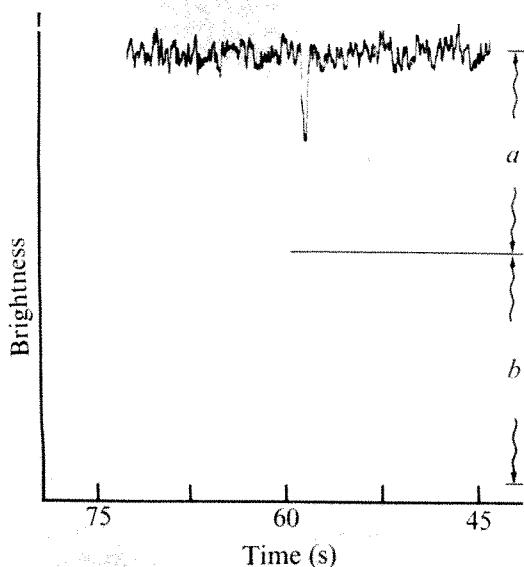


Fig. 1 The occultation light curve observed at Perth for the ϵ ring. The number of seconds past 20 h 10 min 00s is given along the abscissa with time increasing to the left. The sharp drop in signal following the occultation resulted from an interruption of the observations to check the centering of the planet in the photometer's entrance aperture. The contributions to the total signal by SAO 158687 and Uranus are indicated by *a* and *b* respectively.

in circular orbits in the equatorial plane, their orbital radii are approximately 42,000 and 42,250 km.

The Perth light curve of the occultation by the ϵ ring is shown in Fig. 1. This is by far the longest of the five occultations observed at Perth, with the ring extending for a distance of about 90 km along the occultation chord. The abruptness with which the occultation begins and ends indicates that the ring has rather sharp boundaries. Substantial variation in transparency across the ring is seen. Since at no point was the star totally obscured, it can be concluded that the majority of the individual particles in the ring have dimensions smaller than the apparent diameter of the star at Uranus, which we estimate to have been about 4 km. The maximum obscuration which we observed corresponds to an optical thickness of about 2.3,

Fig. 2 The occultation light curve observed at Perth for the γ ring. The number of seconds past 20 h 15 min 00s is given along the abscissa with time increasing to the left. The contributions to the total signal by SAO 158687 and Uranus are indicated by *A* and *B* respectively.



which is substantially larger than that of the B ring of Saturn⁷.

Figure 2 shows the occultation light curve observed at Perth for the γ ring. The very brief duration of this occultation implies that the width of the ring is no more than a few kilometres. The other three occultation light curves observed at Perth are very similar in character to that produced by the γ ring.

Further occultation observations have been reported^{8,9}.

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A new satellite of Uranus

WHILE observing the occultation of the star SAO 158687 by Uranus on 10 March 1977 we came across an unexpected event suggesting the existence of a hitherto unknown satellite of the planet. The observations were carried out at the Kavalur Observatory (lat. 12° 34' 32" N; long 78° 49' 54" E) using a near infrared photometer consisting of a refrigerated EMI 9558B phototube and Kodak Wratten 89B filter attached to the Cassegrain focus of the 102-cm telescope. The individual deflections due to the star and the planet were measured during the previous night, as well as a few hours before the predicted instant of occultation. A 16-arc s diaphragm was used during occultation, whereby the planet and the star could be conveniently contained for several hours around the epoch of occultation. Guiding and monitoring of the objects were done through the 20-cm guide telescope, with periodic checking of the centering in the main telescope. As indicated by the prediction¹, we expected only a grazing occultation to be visible at Kavalur, resulting in a slight diminution in the intensity of starlight owing to the extinction effect by differential refraction in the outer layers of the planet's atmosphere². A very shallow depression of 0.046 magnitudes in the light of SAO 158687 was indeed observed.

About 40 min before the midpoint of this dip we observed at 20 h 19 min 38 s UT a sharp drop in the light curve which lasted 8.9 s (Fig. 1). The phenomenon was visually observed in the guide telescope also, as a sudden disappearance of the star in the field of view followed by an equally sudden reappearance, a few seconds later. Taking the most recent positions of Uranus and that of the star (C. Lecacheux, personal communication), this point is found to be 4".1 away from the centre of the planet at a position angle of 349°. Assuming a circular orbit and correcting for foreshortening due to orientation of the axis of the orbit (assumed to be same as that of the planet), we arrive at a value of 53,000 km for its radius, and an approximate period of 9 h 30 min. The apparent motion of this satellite's shadow with respect to the observer is

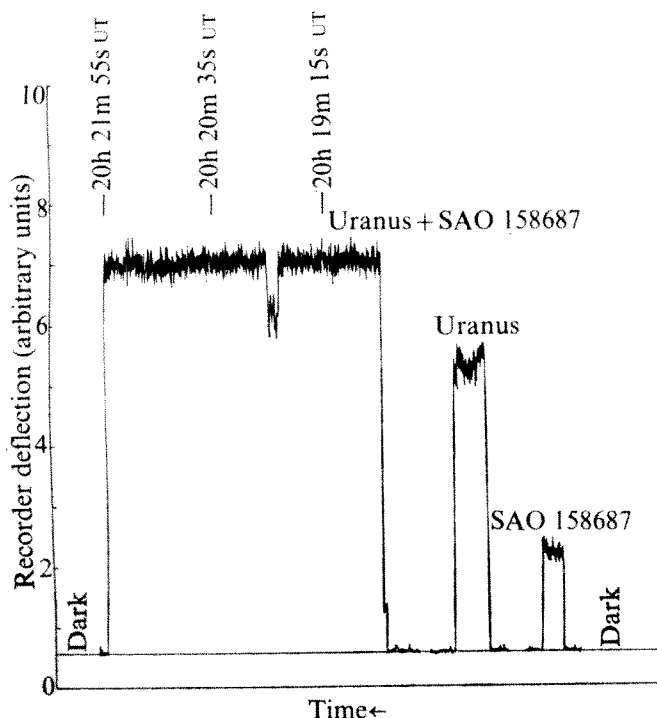


Fig. 1 Light curve obtained during occultation of SAO 158687 by Uranus.

estimated to be 2.74 km s^{-1} , yielding the length of the chord joining the immersion and emersion points as 24 km.

The size of the body can be estimated from the occultation light curve, where the dip is only 53% of the total star light. This can be explained by postulating that the stellar disk subtends an angle comparable to that of the satellite and the occultation in partial. We assume a typical stellar diameter of 3.5×10^{-4} arc s for a K5 star with the appropriate distance modulus³. Then, from a model of the occultation geometry, and taking into account the diffraction near uniform edges, we find that the estimated diameter will be around 70 km. The visual magnitude of the satellite is estimated to be ~ 19 .

We conclude that the body is a new satellite of Uranus.

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Spectral variability in X-ray sources

COE *et al.*¹ have reported that Cyg X-1 and the transient source A 0620-00 both display anticorrelated spectral variability. That is to say, flux enhancements at low energy ($E \lesssim 10 \text{ keV}$) tend to be accompanied by a decline at high energy and vice versa. Both sources may be considered possible black holes; Cyg X-1 is well known, while the candidacy of A 0620-00 ($\equiv$ Nova Monocerotis 1975, distance $\gtrsim 2 \text{ kpc}$)^{2,3} follows if its peak luminosity $\gtrsim 5 \times 10^{38} \text{ erg s}^{-1}$ is identified with the Eddington limit luminosity $1.3 M/M_{\odot} \times 10^{38} \text{ erg s}^{-1}$ for mass accretion. Thus the accreting object has a mass $\gtrsim 4 M_{\odot}$, just greater than the accepted limiting mass for a neutron star. It has therefore been suggested¹ that anticorrelated spectral variability, perhaps resulting from inverse Compton scattering in the accretion

disk^{4,5} may be common to X-ray emitting binary systems which contain black holes. In this paper, we wish to show that anticorrelated variability is not confined to sources which are black hole candidates.

We have examined published spectral data for the above two objects and four other well-observed sources — namely, Cen X-3, Her X-1, Cyg X-3 and Cir X-1. The references to all data used in this paper (more than 50 observations) are too numerous to mention here and are tabulated elsewhere⁶. In order to facilitate comparisons we have selected only those results for which a power law spectrum has been fitted, of the form

$$I(E) = AE^{-\alpha} \text{ (keV cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}) \quad (1)$$

In some cases the power law may not have provided the only fit to data but we assume that the quoted spectral index α characterised the spectral shape at the time of observation and allows ready comparison to spectra at other times.

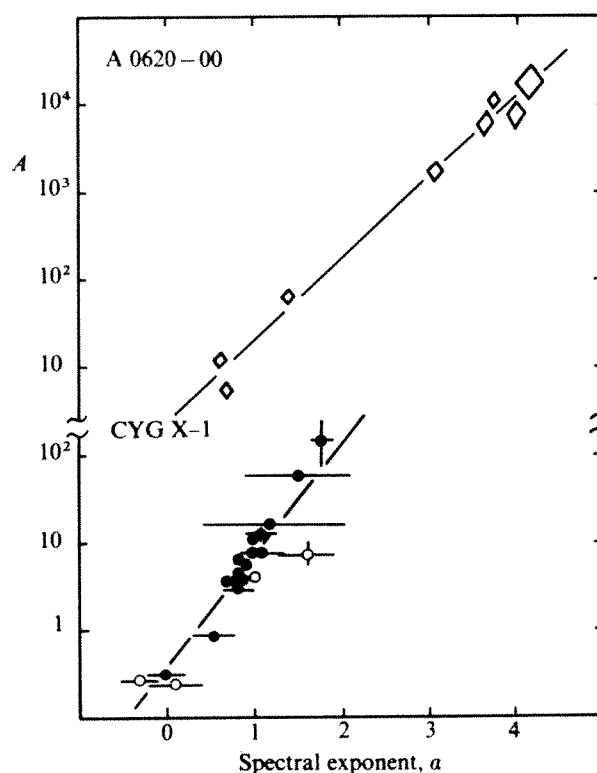


Fig. 1 Spectral variability plots for black hole candidates A 0620-00 (2–15 keV) and Cyg X-1. Open circles: low energy, $\lesssim 30 \text{ keV}$; full circles: high energy, $\gtrsim 30 \text{ keV}$. The lines of best fit are shown.

Spectra displaying anticorrelated variability about a 'pivot' energy E_p are described by $I(E) = A'(E/E_p)^{-\alpha}$ where $A' = \text{constant}$. Comparison with (1) therefore implies that $A'E_p^\alpha = A$, or $\log A = C + S\alpha$, where $S (= \log E_p)$ and C are constant for the source in question. Therefore, in order to better quantify anticorrelated variability we have plotted spectral observations as points on the $(\alpha, \log A)$ plane (hereafter called spectral variability plots, or SVPs), looking for a linear correlation, Fig. 1 shows these data for Cyg X-1 and A 0620-00 and the clearly linear correlations confirm the findings of Coe *et al.*¹. Linear correlation coefficients and the associated negligible probabilities that these correlations could be due to random fluctuations are shown in Table 1. In particular, it is found that $S \sim 1.3$ and 0.9 implying $E_p \sim 20$ and 8 keV for Cyg X-1 and A 0620-00, respectively.

Application of this technique to the other sources (Fig. 2) leads to the surprising result that all four behave in a similar

Table 1 Summary of results

Source	<i>N</i>	$S \pm 2\sigma$	<i>R</i>	<i>P</i>
Cyg X-1	25	1.3 ± 0.2	0.92	< 0.001
A0620-00	8	0.9 ± 0.1	0.99	< 0.001
Cyg X-3	5	1.1 ± 0.3	0.97	0.005
Her X-1	9	1.3 ± 0.3	0.95	< 0.001
Cen X-3	5	0.9 ± 0.5	0.90	0.03
Cir X-1	10	1.3 ± 0.3	0.97	< 0.001

N is the number of observations; *R* is the correlation coefficient; *P* is the probability that the correlation arises by chance.

manner to the black hole candidates. Taken alone the result for Cen X-3 is only marginally significant; nevertheless it produces a value for *S* similar to that for the other sources (see Table 1). In all cases best fit values of *S* lie between 0.9 and 1.3 corresponding to E_p values between 8 and 20 keV. It would appear therefore that all six sources display anticorrelated spectral variability.

We have discriminated between observations made predominantly below and above 30 keV ('low' and 'high' energy) in order to determine if spectral breaks unrelated to time variability have introduced spurious effects. We have sufficient data for Cyg X-1 to allow formal fits to be made with and without the low energy points. No significant difference in the

quality of the fits or in *S* values could be obtained. Low and high energy data points are distinguished for all the sources in Figs. 1 and 2 and we conclude that there is no evidence to suggest that the correlation between *S* and α is caused by energy dependence of the spectral exponent.

This study suggests that anticorrelated spectral variability may occur in X-ray sources other than those favoured as black hole candidates. It will be of interest to see if proposed inverse Compton emission mechanisms which show promise of explaining the observations for the black hole candidates^{4,6} can be generalised for binary systems involving say, neutron stars or white dwarfs.

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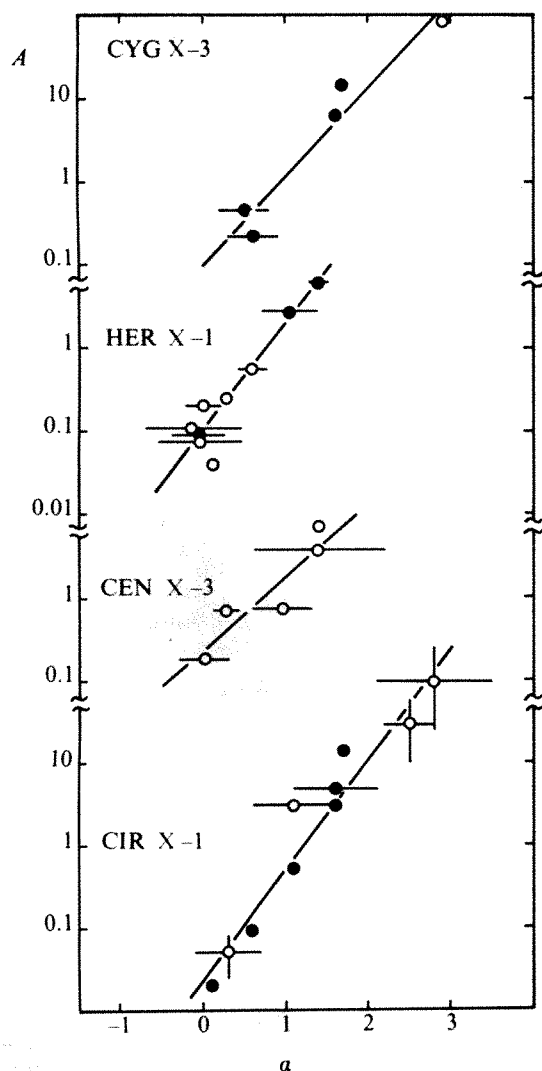


Fig. 2 Spectral variability plots for Cyg X-3; Her X-1; Cen X-3 and Cir X-1. Open and full circles represent low (< 30 keV) and high (> 30 keV) energy observations respectively. The lines of best fit are shown.

Statistical mechanics of microemulsions

At low surfactant concentrations, an oil-water microemulsion may be represented as two immiscible continuum fluids, oil and water, dispersed in one another so as to form two interpenetrating, multiply connected regions, with the surfactant adsorbed at the interface between them. Scriven¹ has pointed out that as the oil/water ratio is increased, three types of structure will evolve in succession: starting from the oil-in-water emulsion to be expected at low oil content, there should be a smooth transition to a bicontinuous structure, in which neither phase can be said to surround the other, followed by a second transition to a water-in-oil emulsion when the oil content is sufficiently high. It seems likely, as Scriven suggests, that the phase diagrams reported for microemulsion systems involve all of these structures. Here we propose a statistical mechanical model for microemulsions, based on a simple procedure for generating random two-fluid geometries. Over certain ranges of the model parameters, the resulting free energy-composition surface shows regions where a bicontinuous phase may coexist with an oil-in-water and a water-in-oil emulsion; such three-phase equilibria have been observed experimentally². Our calculations also suggest a possible explanation of the very low interfacial tensions sometimes found between microemulsion phases.

To determine, at least within a constant of proportionality, the number of configurations available to the oil and water regions in a microemulsion of specified composition, we restrict ourselves to the class of geometries resulting from a two-step process, consisting of a Voronoi tessellation³⁻⁵ followed by random segregation into oil and water domains. *N* points are first distributed completely at random over the volume *V* to be occupied by the microemulsion; with each of these Poisson points, we associate the polyhedral region which lies closer to it than to any of the other (*N* - 1) points (Fig. 1). In the second step, *N*_o of these polyhedra are selected at random to be occupied by oil, and the remainder filled with water; the result is a random two-fluid geometry in which the oil occupies a fraction $v_o = N_o/N$ of the total volume.

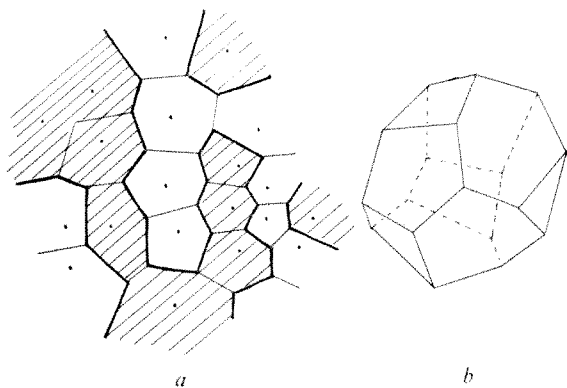


Fig. 1 *a*, Two dimensional representation of the partition process. Shaded regions are occupied by oil, unshaded regions by water; heavy lines indicate the surfactant monolayer. *b*, Typical Voronoi polyhedron.

The number of distinct positions available to a Poisson point is proportional to the volume V , the proportionality constant $1/\omega$ being one of the model parameters. It is the size of ω that determines the scale of the microemulsion structure, and it is through ω that information about the molecular nature of the water and oil fluids enters into the model. The number of configurations for the placement of all N points is then

$$(V/\omega)^N/N!$$

and for each configuration there are

$$N!/N_0!(N-N_0)!$$

ways of assigning the oil and water labels. The configurational entropy of the microemulsion is therefore

$$S = -Vkc(\ln(\omega c/e) + v_0 \ln v_0 + (1-v_0) \ln(1-v_0)), \quad (1)$$

where $c \equiv N/V$ is the concentration of Poisson points.

To translate equation (1) into predictions about the thermodynamic behaviour of the system, we need an expression for the surfactant concentration σ in terms of c and v_0 . We assume that all of the surfactant is located in a monolayer at the interface between the oil and water regions, and that there are no oil-water contacts unmediated by such a monolayer. The surfactant concentration thus fixes the oil-water interfacial area.

The total area per unit volume of the surface generated by the Voronoi tessellation³ is $2.91c^{1/3}$, and the total length of the polyhedral edges in unit volume is $5.83c^{2/3}$. Only a fraction $2v_0(1-v_0)$ of the total surface will actually separate oil from water polyhedra; furthermore, since each edge is shared by three polyhedra, the probability that it lies on the oil/water interface is $3v_0(1-v_0)$.

We assume that a plane interface between the oil and water regions adsorbs α moles of surfactant per unit area, but that a curved surface will, because of crowding of surfactant segments, have a somewhat lower surfactant capacity. This curvature effect is introduced into the model by lowering the amount of adsorbed surfactant by β moles per unit edge length. The expression for σ derived from these considerations is

$$\sigma = (5.82\alpha c^{1/3} - 17.5\beta c^{2/3})v_0(1-v_0) \quad (2)$$

Equations (1) and (2) together may be used to determine the configurational free energy $G = -TS$ as a function of σ and v_0 (in calculating c for a given value of σ , we use the lower of the two roots of equation (2)). If the results are expressed in reduced quantities

$$G^* = \omega G / e V k T, \quad \sigma^* = \omega^{2/3} \sigma / 17.5 e^{2/3} \beta \\ \alpha^* = \omega^{1/3} \alpha / 3.0 \beta e^{1/3}$$

the reduced free energy G^* will depend only on σ^* , v_0 , and α^* ; the shape of the free energy surface, and therefore the phase equilibria to be expected, are entirely determined by the dimensionless parameter α^* .

Figure 2*a* shows plots of G^* against v_0 for various values of σ^* at $\alpha^* = 2.0$; below $\sigma^* = 0.247$, the plots show two symmetrically placed minima, the locations of which represent the compositions of two microemulsion phases in equilibrium. In Fig. 2*b* we have plotted (schematically only, in order to show the region of negative curvature) the minimum values of G^* from Fig. 2*a* against σ^* . The two compositions at which the double tangent touches the curve correspond to a bicontinuous phase ($\sigma^* = 0.247$, $v_0 = 0.5$) in equilibrium simultaneously with two other microemulsions of lower surfactant content ($\sigma^* = 0.102$, $v_0 = 0.121$, and $\sigma^* = 0.102$, $v_0 = 0.879$)—in other words, a three-phase equilibrium.

Figure 3 shows the three- and four-phase domains in the three-component phase diagram for various α^* : a mixture whose overall composition is located in the interior of a triangle or quadrilateral separates into phases with compositions given by the vertices; for α^* values outside the range 1.63–3.5 only two-phase equilibria are possible. The apparent violation of the phase rule by the four-phase regions found below $\alpha^* = 1.75$ results from the symmetry between the oil and water components imposed by our model. The slightest perturbation of this

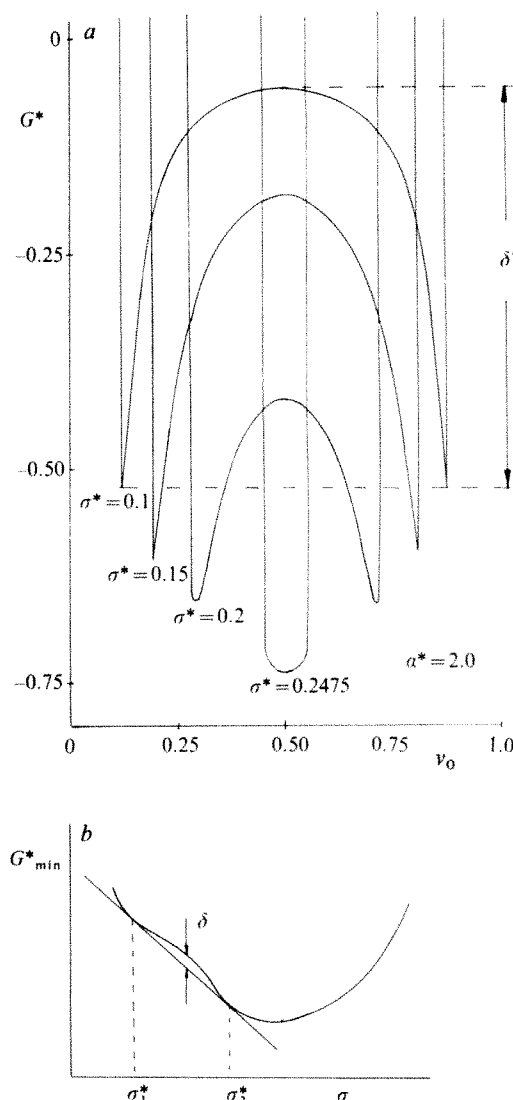


Fig. 2 *a*, Reduced free energy plotted against volume fraction of oil at $\alpha^* = 2$ and several values of σ^* . *b*, Schematic plot of minimum free energy against σ^* .

symmetry leads to replacement of the top and one side of a four-phase quadrilateral by the corresponding diagonal; the resulting triangle represents a three-phase equilibrium.

In addition to predicting, at least qualitatively, the existence of the multiple phase equilibria observed experimentally, our model also suggests the origin of the very low interfacial tensions between the separated microemulsion phases observed for certain systems². In Fig. 2b the maximum difference δ between the G^* curve and the double tangent is 0.0008, to be compared with a difference $\delta' = 0.47$ between the reduced free energies for the corresponding homogeneous and phase-separated mixtures in Fig. 2a. Although no quantitative calculations have yet been made, the implication is strong that a very thin layer of the bicontinuous phase can reduce the interfacial tension between a water-in-oil and an oil-in-water microemulsion by several orders of magnitude.

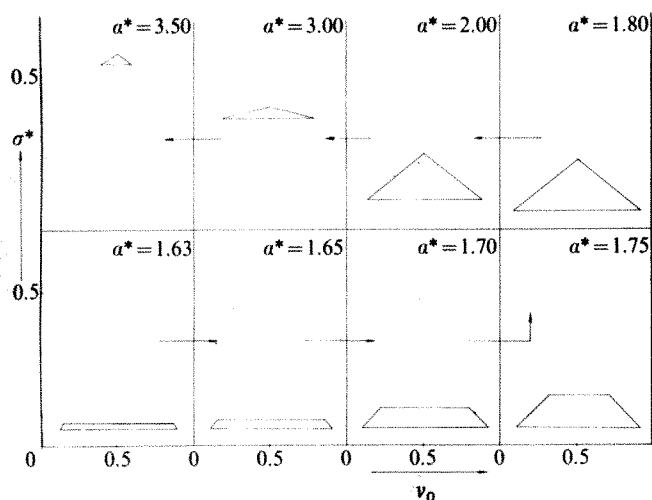


Fig. 3 The three- and four-phase regions at various values of α^* .

Our aim has been to offer a minimal model of microemulsion phase equilibria, a model which leads to a purely entropic free energy function. In the absence of composition-dependent energy contributions, all phase equilibria should be independent of temperature, in apparent contradiction to experimental fact. It should be remembered, however, that the model assumes an ideal surfactant, one which exists only at the water/oil interface. The balance between hydrophilic and lipophilic interactions required for such optimal performance will in general be quite sensitive to temperature changes, particularly in the case of the surfactant-cosurfactant systems used in practice.

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Toxic fluorinated compounds as by-products of certain BF_3 -catalysed industrial processes

WE have embarked on a programme of toxicity tests on a range of industrial chemicals that are manufactured using boron trifluoride (BF_3) as a catalyst. Although this study is still in its early stages, we feel it opportune to draw attention to the fact that in some of the samples we have detected low concentrations of fluorinated compounds, some of which merit serious toxicological concern. The most notable example we have uncovered concerns some industrial chemicals that are manufactured from ethylene oxide using BF_3 as catalyst. On the basis of the results of toxicological studies of these products, we suspected fluoroacetate precursors as contaminants, and subsequent chemical analysis has confirmed the presence of low concentrations of 2-fluoroethanol and/or 2-(2-fluoroethoxy) ethanol. These two compounds are known to be very toxic to mice (LD_{50} 10 and 15 mg kg^{-1} respectively) and are regarded as fluoroacetate precursors^{1,2}. The latter is the addition product of 2-fluoroethanol and ethylene oxide (equation 1), and 2-fluoroethanol is the formal addition product of fluoride ion to ethylene oxide (equation 2).



This explanation of the genesis of the two fluoroacetate precursors points, not to BF_3 itself, but to fluoride ion as the source of the toxicants. That F^- is a common by-product of BF_3 -catalysed reactions is further supported by the identification of tetrafluoroborate ion (BF_4^-) as a contaminant of some of the other samples we have examined. For this chemical is the readily-formed product of interaction between F^- and BF_3 . The origin of fluoride ion is unclear; it might derive by solvolysis of BF_3 —or of silicon tetrafluoride, SiF_4 , a known contaminant of commercial BF_3 .

Fuller details of these studies will be published elsewhere.

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Aragonite in Permian Reefs

PRESERVATION of aragonite in fossils decreases the further we go back in the geologic record. In the Palaeozoic, aragonitic remains have been found only in clayey-bituminous deposits which have largely inhibited diagenetic alterations of unstable mineral phases (for example, Buckhorn Asphalt and Kendrick Shale, both Upper Carboniferous of USA¹, and Upper Oil Shale Group, Lower Carboniferous of Scotland²). Palaeontological information preserved in these facies is restricted, however, to those organic remains which lived in the equivalent basinal environments or were transported into them. Original mineralogy and microstructures of ancient reef organisms can generally only be deduced from analogies with equivalent younger counterparts. The oldest known reef-organisms with preserved aragonitic microstructures were

Table 1 Microstructure, cement and aragonite contents in typical Permian reef organisms of Djebel Tebaga (southern Tunisia)

Specimen no.	Determination	Microstructure of the skeleton	Cement	% of aragonite
T 31/6	Solenoporaean alga	Recrystallised	Blocky calcite	37
T 31/7	<i>Stellispongia bacilla</i> Termier & Termier	Spherulitic	Blocky calcite	11
T 20/6	<i>Stellispongia</i> cf. <i>lobata</i> (Parona)	Recrystallised	Locally acicular aragonite, mostly blocky calcite	20
T 31/8	<i>Stellispongia</i> sp.	Irregular	Blocky calcite	20
T 7/1	<i>Disjectopora</i> sp.	Spherulitic (?)	Acicular aragonite + blocky calcite	19
T 26/1	Chaetetid sclerosponge	Spherulitic	Blocky calcite	33
T 209	Chaetetid sclerosponge	Spherulitic	Blocky calcite	21
T 7/2	<i>Plerophyllum</i> (<i>Plerophyllum</i>) sp.	Recrystallised	Blocky calcite	—
T 31/10	<i>Permosoma permotessellata</i> (Parona)	Clinogonal	Blocky calcite	6.5

found in the Upper Triassic (Lower Carnian) of the Southern Alps³. Here, early burial of patch reefs and reef detritus by clayey-tuffaceous sediments and porosity diminution of the carbonates by subsequent cementation have probably inhibited transformation of aragonitic microstructures into calcite⁴. In this report we describe investigations of microstructures and diagenesis in calcareous sponges which have revealed aragonitic primary structures and cements in material from the Upper Permian of Djebel Tebaga, Southern Tunisia.

The Upper Permian specimens were collected from small patch reefs and their marginal reef detritus, intercalated in an irregular sequence of sandstones and marls, crinoidal, brachiopod and fusulinid limestones⁵. Calci-sponges (Inozoa, Sphinctozoa) and sclerosponges are the main reef-builders. They are associated, especially in the marginal reef areas, with stromatolites and solenoporaean algae. The enigmatic tabulate coral-like fossil *Permosoma permotessellata* (Parona) is, in places, a successful frame-builder. Rugose corals, so close to their presumed extinction

on the Permian-Triassic boundary, have been found only in two places and do not contribute to reef construction. The marginal areas of the patch reefs and the surrounding reef detritus have yielded the greatest number of individuals and species. Externally the specimens are rather well preserved, beautifully washed out by recent erosion, but appear highly altered by diagenesis. Calcareous sponges are often partly or totally dolomitized.



Fig. 1 *Disjectopora* sp., Upper Permian, Djebel Tebaga (Tunisia). T 7/1. Vertical thin section through astrorhizal canal with two tabulae, showing spherulitic aragonite cement along the outer margin and blocky calcite in the centre of the voids. Primary microstructure of the skeleton obliterated by recrystallisation. Bar represents 100 μ m.



Fig. 2 Chaetetid sclerosponge, Upper Permian, Djebel Tebaga (Tunisia). T 209. Spherulitic microstructure of the skeleton, partly recrystallised. Bar represents 10 μ m.

The occurrence and distribution of aragonite was first shown by staining thin sections with Feigl solution. Thirty specimens, mostly calcareous sponges (pharetronids, sclerosponges), a few corals, calcareous algae (solenoporaean) and *Permosoma* were X-rayed for determination of calcite, aragonite and dolomite concentrations. In 20 samples aragonite contents were between 1% and 37%; 10 samples were free of aragonite, and in three samples dolomite contents were up to 36%. Aragonite is patchily distributed in the massive skeletons of the above groups and/or as a thin veneer on the skeletal elements. This veneer is the first generation of cement, with acicular shaped crystals which were often translucent (Fig. 1). The preservation of organic microstructures does not necessarily improve with an increase in aragonite content. As can be seen from Table 1, even with complete recrystallisation of the original crystal fabric, the remaining aragonite content may reach 20%. As has already been stated for Triassic calcareous sponges³, the occurrence of certain microstructures is not a consistent generic feature of calcareous sponges. Representatives of *Stellispongia*, for instance, which exhibit

clinogonal or irregular arrangements of aragonite crystals in the Triassic, have spherulitic microstructures in Permian species: the latter fabric is the most common in Permian calcareous sponges, having been encountered in inozoans⁶, sphinctozoans⁷, sclerosponges (Fig. 2) and stromatoporoids (disjectopores). Clinogonal microstructure has been found in one specimen of *Stellispongia*, and the tube-walls of *Permosoma permotessellata* (Parona). Tabulae and opercula of this species show irregularly spherulitic structure⁶.

The surprising preservation of aragonitic organic microstructures and cements in the Permian reef deposits of Tunisia is being subjected to further mineralogical and geochemical investigations. Material so far examined demonstrates that even 'hopeless' facies with obviously complete diagenetic alteration may still retain a large amount of mineralogical and microstructural information.

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The Grenville Province as a shear zone

PALAEOMAGNETIC and structural data on the Grenville Province in North America are best explained if the Grenvillian 'orogeny' was caused by right-lateral simple shear of the entire Province. Relative displacement of the edges of the belt is estimated at 200–300 km.

Apparent polar wander paths (APW) for the Grenville Province are clearly distinct from the North American path at the corresponding time (Fig. 1). This abnormal distribution has been explained by a collision between the Province and the rest of the continent^{1–3} or by an extra loop of the North American APW path⁴. Relative ages of magnetisation from minerals that have given two distinct polar positions would all seem to indicate however that the collision model is palaeomagnetically more satisfactory. Geological evidence, on the other hand, militates against the existence of a suture anywhere in the Grenville Province. The model thus requires that the postulated suture be hidden under the Appalachians, and that the Grenville Province be an ancient analogue of the modern Tibetan plateau^{1,5}.

Another hypothesis that satisfies palaeomagnetic constraints is that the Grenville 'track' formed by counter-clockwise rotation of individual structural elements inside the Grenville Province. Samples that show two directions of magnetisation separated by very large angular distances come from severely deformed areas, whereas individual poles closest to the normal position for North American poles at that time come either from rocks close to the Grenville Front or from highly resistant, moderately deformed zones. By contrast, poles from samples collected in strongly deformed belts, or far from the Grenville Front plot farther away along the Grenville track (Fig. 1). The length of this track suggests that samples from intensely deformed rocks may have suffered up to 60° of counter-clockwise rotation relative to other North American poles.

This interpretation of palaeomagnetic data makes it worth re-examining the structure of the Grenville Province. A

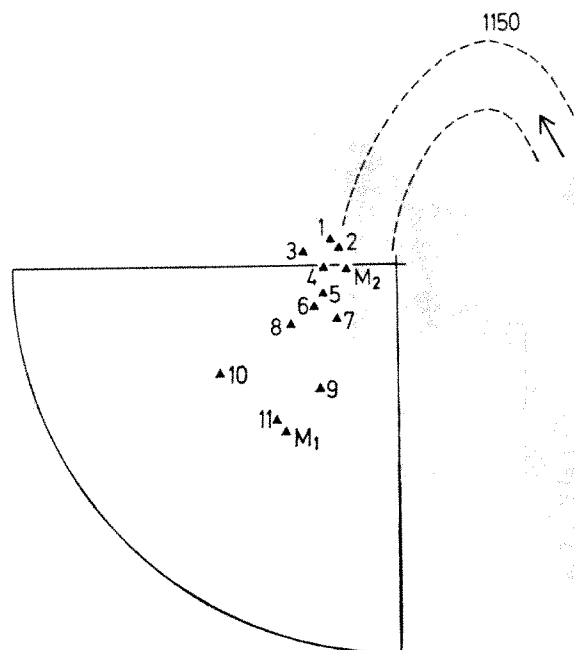


Fig. 1 Stereographic projection of palaeomagnetic pole positions from the Grenville Province. Michael gabbro (1); Grenville Front anorthosite (2); Shabogamo gabbro (3); Unfraville gabbro (4); Lac St Jean anorthosite (5); St Urbain anorthosite (6); post-Grenville Frontenac dikes (7); Wilberforce pyroxenite (8); Ottawa diorites (9); Magnetawan gneisses (10); Lac Allard anorthosite (11); M₁ and M₂, two pole positions from the Morin anorthosite. The apparent polar wander path for North America between 1150 and 950 Myr BP is sketched in.

northeasterly trending shear zone closely follows the Grenville Front⁶ and another, less developed, parallel belt marks the southeastern margin of the Province (Fig. 2). Between these two shear zones, the Province is broken into a number of blocks separated by north-northeasterly trending shear belts—'straight belts'⁷ such as the Chibougamau–Gatineau lineament⁸—that, in numerous cases, are underlined by mylonites. This pattern, visible in Fig. 2, is also present at larger scales.

Although strain measurements in the field show predominant flattening and/or constriction, deformation in straight belts must occur at least in part by simple shear at the scale of the belt. Rigid blocks of small dimensions are represented by granitic plutons and anorthositic massifs, whereas larger ones seem to correspond to fragments of an older basement that have escaped the brunt of Grenvillian deformation. Shear belts tend to follow strips of metasediments (marble and paragneiss of the Grenville Group) but also appear to have remobilised the basement where its pre-existing structures were suitably oriented. Where the map pattern indicates relative displacements along the edges of blocks, they correspond to counter-clockwise rotation of the blocks. Their rotation is thus antithetic to that of the Grenville belt as a whole. The structural pattern is thus everywhere compatible with deformation of the Grenville Province by overall simple shear (Fig. 2).

According to geochronological data, the Grenvillian event did not start much before 1150 My ago and was over by about 950 My ago. During this interval, the entire region was heated sufficiently for all K–Ar clocks to be reset. Rb–Sr isochron dates have also been reset in the Chibougamau–Gatineau shear belt, but this was not the case in the more rigid block located to the west of it.

By analogy with Labrador, anorthositic suites are commonly thought to be about 1400 Myr to 1500 Myr old, but Rb–Sr and U–Pb dates on the Adirondacks⁹, Morin¹⁰ and Lac St Jean complexes¹¹ are Grenvillian. Low ⁸⁷Sr/⁸⁶Sr initial ratios and apparent syntectonic emplacement of the

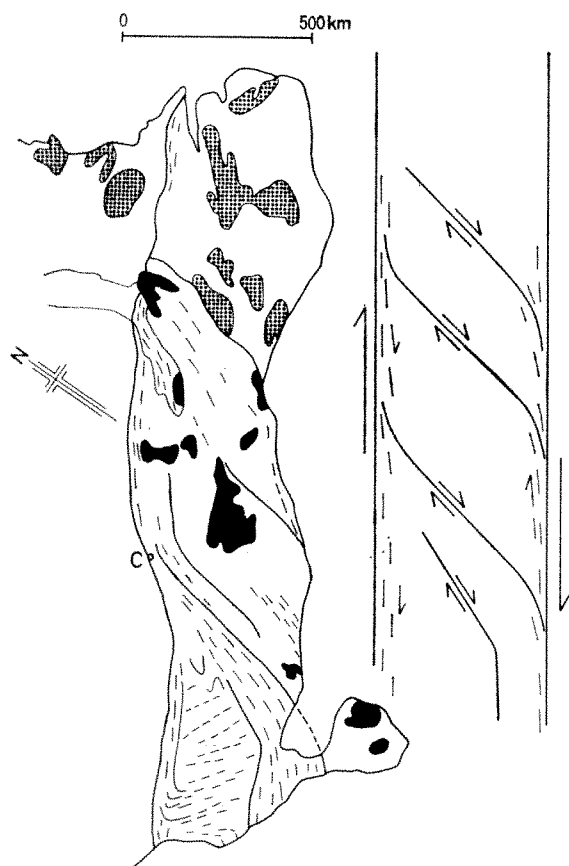


Fig. 2 Sketch of the Grenville Province and suggested shearing mechanism. Thicker continuous lines indicate boundaries of subprovinces; thin dashed lines show structural trends. Anorthosite massifs with mafic roots are dotted in, those without mafic roots are black. C, Chibougamau on the Grenville Front, at the north end of the Chibougamau-Gatineau lineament.

Morin pluton¹² indicate that those three anorthositic complexes are coeval with the Grenvillian deformation. In fact, the peculiar shape of the Morin massif may result from clockwise rotation of this pluton during its emplacement. The lack of a mafic root in all three massifs, by contrast with those of eastern Labrador, probably reflects syntectonic emplacement.

Numerous field studies have demonstrated the synchronicity of deformation and metamorphism in the Grenville Province, so that some heat source must have been available during deformation. Without this excess heat, it is indeed doubtful that such a wide zone (~300 km) would have been deformed by simple shear. The 1500 Myr old anorthositic suite occurs along a small circle that is in close geographical proximity to the Grenville Province. It is possible that the same deep-seated hot belt responsible for these plutons may have underlain the Grenville Province around 1,100 Myr to 1,000 Myr ago. As the Province follows a small circle of about 60° radius, one may envisage its deformation to have corresponded to clockwise rotation relative to North America around an Eulerian pole located at the centre of the circle. In more general terms, this style of deformation appears to be characteristic of Proterozoic mobile belts including the Pan-African ones¹³⁻¹⁵.

From apparent displacement of possibly equivalent rocks, the sum of right-lateral strike-slip motion across the Province is about 200-300 km, a figure comparable to those derived from apparent rotation of palaeomagnetic poles.

In this interpretation, the Grenville Front can be at various points a fault, a thrust or a metamorphic boundary, depending upon the location and the orientation of its

various segments relative to the overall shear direction. The synchronous opening of the Keweenaw graben is taken to represent a fracturing of the old brittle Archean craton, caused by right-lateral shear in the Grenville Province, whereas the intrusion of the Mackenzie diabase dyke swarm represents the tension phase that preceded and accompanied deformation in the Province¹⁶.

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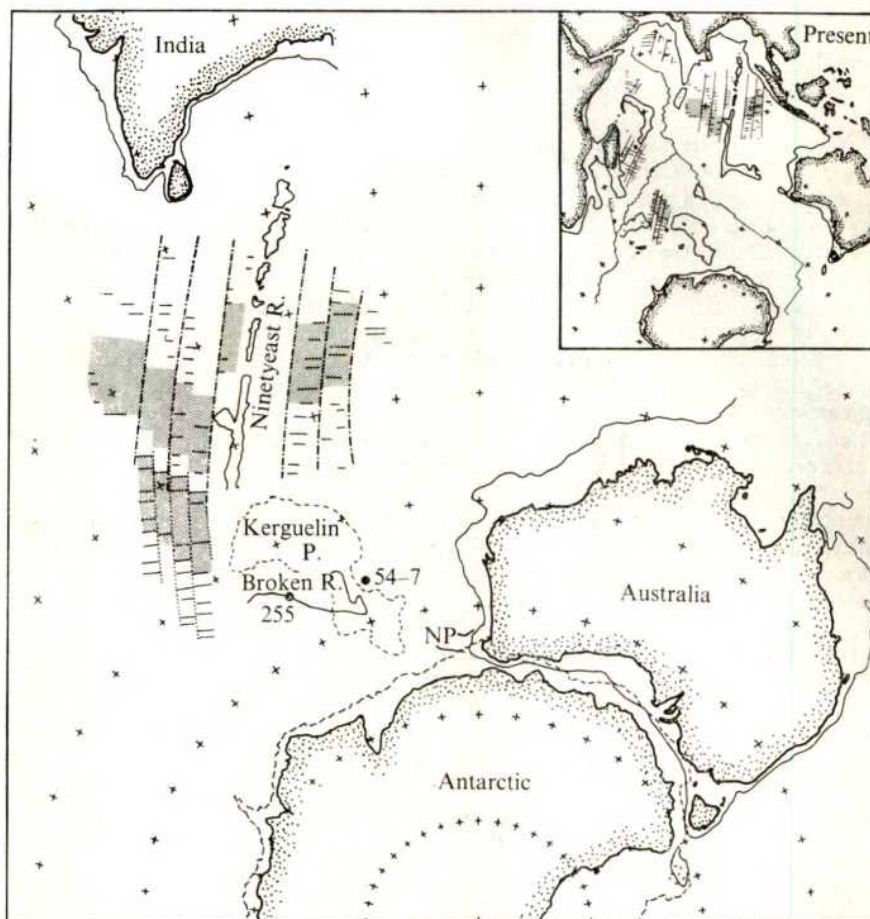
Implications of a revised fit between Australia and Antarctica for the evolution of the Eastern Indian Ocean

GRIFFITHS¹ suggested that the computer fits of Australia and Antarctica²⁻⁴ are not supported by geology and that a better fit can be obtained by moving Australia 250 km westwards along the coast of Antarctica in the computer fitted reconstructions. Griffiths's revised fit eliminates the overlap of the South Tasman Rise, now known to be of continental origin⁵, and the Iselin plateau. Laird *et al.*⁶ showed that new geological evidence from Tasmania and East Antarctica favour such a revised reconstruction over the older computer fits. We present a slight modification to the fits given by Griffiths and by Laird *et al.* that, in addition, allows a good fit of magnetic anomaly lineations and fracture zone trends on the India⁷ and Antarctic⁸ plates (Fig. 1). This revised reconstruction thus implies that India and Australia have been parts of the same plate since the time of anomaly 22 (about 50 Myr ago). It also substantially reduces the size of the postulated Greater India⁹⁻¹¹ (Fig. 2).

Figure 1 shows this reconstruction of the eastern Indian Ocean at anomaly 22 time. Australia is rotated to Antarctica by 30.9° about a pole at 11.9°N, 30.8°E. India is attached to Australia and rotated with it in Fig. 1. Magnetic lineations and fracture zones^{7,8,12} are also shown, with anomalies 23 to 28 shaded. The fit was done by eye, using the geological data⁶ as a constraint and the 2,000-m isobaths as the continental edges. There is some overlap in West Australia (also present in the computer fits²⁻⁴), and a gap in the Australian Bight, but the fit in the eastern sector is good.

As in the computer reconstruction of McKenzie and Sclater⁴, there is a large overlap of Kerguelen Plateau and Broken Ridge. Calcareous sediments of Cretaceous age have been recovered from both these features^{13,14} which indicates that they were elevated structures before Australia and Antarctica separated. Broken Ridge is an elongate tilt-block structure¹⁵ with a steep southern slope dropping into the Diamantina fracture zone. Kerguelen Plateau has been poorly mapped, but in outline it does seem to divide along approximately 55°S into northern and southern sections.

Fig. 1 Reconstruction of eastern Indian Ocean at anomaly 22 time. Antarctica fixed. Isobaths are 2,000 m for Australia and Antarctica (dashed), 3,000 m for Kerguelen Plateau (dashed), Broken Ridge and Ninetyeast Ridge north of inferred position of anomaly 22 (ref. 7). DSDP site 255 on Broken Ridge¹³ and the site of Eltanin core 54-7 (ref. 14) are shown. NP, Naturaliste Plateau. Equal area projection, 10° grid spacing.

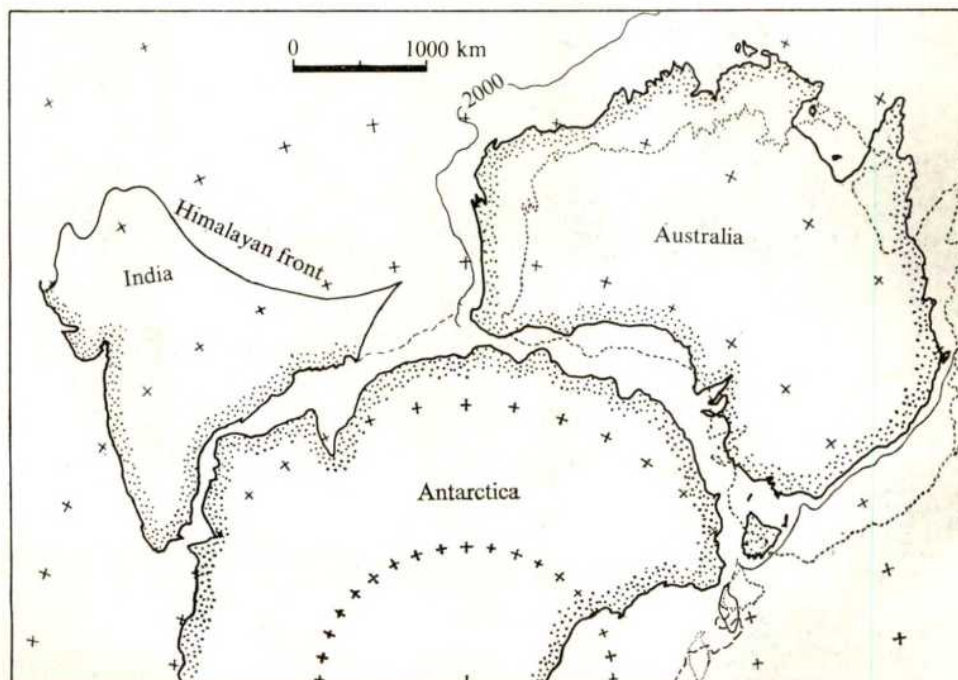


Kerguelen and Heard Islands on the northern section have been the site of recent volcanism¹⁶ although early Tertiary limestones occur on Heard Island¹⁷. Cenomanian chalk was cored from the flanks of the deeper southern section¹⁴. We suggest, therefore, that the northern section of Kerguelen Plateau has been formed since the Eocene, and that the southern part is a remnant of an older structure, possibly

a ridge that consisted of Broken Ridge, the southern section of Kerguelen Plateau and Naturaliste Plateau.

India moved rapidly northwards away from Antarctica from the late Cretaceous until approximately the time of anomaly 22 when the rate and direction changed markedly^{4,7,8}. At approximately this same time, Australia broke away from Antarctica¹⁸. The reasonably good match

Fig. 2 East Gondwanaland with India in the computer fit² with a computer fit⁴ of Australia as a dashed line.



of the fracture zones and magnetic anomalies on the India and Antarctica plates in Fig. 1 implies that at the time of anomaly 22, India and Australia became joined together as parts of the same plate and motion on the Ninetyeast transform fault ceased.

Sclater and Fisher⁷ in their model for the evolution of the eastern Indian Ocean used a computer fit⁴ between Australia and Antarctica for their reconstructions. This left a longer distance between the sequences of anomalies 22–32 on the India and Antarctica plates than in Fig. 1. As India and Australia are now on the same plate, Sclater and Fisher were forced to maintain a three-plate configuration between the times of anomalies 22 and 11 with right lateral strike-slip motion on the Ninetyeast transform fault. Thus the reconstruction in Fig. 1 suggests that this complexity is unnecessary.

Because the west coast of Australia seems not to have been open to the sea until the Jurassic¹⁹, several authors have suggested that the original Indian continental fragment extended to the west coast of Australia^{9–11}. The revised Australia–Antarctica fit brings Australia approximately 250 km closer to the reconstructed position of India and reduces the extent of 'Greater India' by this amount (Fig. 2). In this new reconstruction, the width of the portion of 'Greater India' between the locus of the present Himalayan Front and the margin of Western Australia is about 600–700 km. As the Himalaya are about 300 km wide and crustal shortening of about this much is suggested by geological and geophysical observations^{20–22}, we contend that essentially all of this Greater India could be absorbed in the Himalaya without the need for postulating the subduction of a large continental fragment beneath Tibet^{9–11}.

Thus the revised reconstruction of Griffiths¹ and Laird *et al.*⁶ not only improves the geologic fit between Australia and Antarctica but allows for a simpler model for the evolution of the east Indian Ocean⁷ and for a smaller amount of material to be absorbed in the Himalaya than had been supposed^{9–11}.

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Sensitivity of the homing pigeon to an Earth-strength magnetic field

THROUGH the use of a flight tunnel constructed within a controlled magnetic environment, it has been possible to train three pairs of homing pigeons (*Columba livia*) to discriminate between the presence and absence of a 0.5 gauss (Earth-strength) magnetic field. Successful discrimination was associated with flutter activity of the birds within the tunnel.

The ability of homing pigeons to return from an unfamiliar release site may depend upon a system of 'map and compass' navigation^{1–3}. Celestial sources of compass information have been demonstrated with pigeons^{4,5} and migratory birds^{6,7}. Sources of map information, however, have remained obscure^{2,3,8}. The occurrence of homing under a wide variety of experimental and natural conditions suggests that several environmental cues are involved.

Characteristics of the Earth's magnetic field, if detected, could participate in the formation of a complete navigational grid. Both the total field strength and the vertical field component (angle of inclination) are maximum at the poles and minimum at the magnetic equator. The angle of declination (between the geographic north and magnetic north meridians) shows complementary variation with longitude.

Early field tests of magnetic orientation were conducted by comparing the homing performance of birds with attached bar magnets to birds with attached non-magnetic equivalent weights⁹. Conflicting results were obtained under sunny test conditions, and no conclusions could be drawn.

Through experiments in which the birds' internal biological clocks were reset, it was found that the Sun provided only compass information^{4,5,10}. Thus, a new series of magnet experiments was prompted by the demonstration of Keeton¹¹ that pigeons could orient and home from an unfamiliar location under complete overcast by using Sun-independent cues. The vanishing bearings of experienced birds released with magnets under overcast were found to be randomised¹². In sunny conditions, however, only experienced birds released at greater distances or first-flight youngsters were disoriented. With a pair of battery-powered Helmholtz coils, Walcott⁴ was able to induce more uniform vertical magnetic fields through the pigeons' heads. By reversing the battery polarity, it was possible to change the inclination of the resultant field and obtain predictable and reversible orientation responses¹³. Normal fluctuations of less than 70 nT (1 gamma = 10⁻⁹ T) in the Earth's magnetic field have also been shown to correlate with the orientation performance under Sun of both pigeons¹⁴ and Singbilled gulls¹⁵. In pigeons, attached magnets have been used to suppress the correlation¹⁶. Walcott (private communication) has found the bearings under Sun of birds released near natural magnetic anomalies to be randomised. Together, these observations suggest that the Earth's magnetic field has a role in the normal navigation of homing pigeons.

With specially designed cages, it has been possible to record the orientation preferences of migratory birds in response to various environmental cues. W. and R. Wilt-schko^{17,18} and Emlen *et al.*¹⁹ have obtained predictable and biologically appropriate responses from several species of birds to natural and artificial magnetic stimuli. Attempts thus far to record the meaningful orientation of pigeons within cages have been unsuccessful, however, and other behavioural techniques have been applied. Using classical conditioning of the pigeon's heart rate, Kreithen and Keeton have demonstrated a sensitivity to polarised light²⁰ and to small changes in barometric pressure²¹. Reille²² reported some positive results in response to magnetic

stimuli; however, Kreithen and Keeton²³, Beaugrand²⁴, and Bookman (unpublished observations) have been unable to repeat or extend his findings. Attempts by several investigators to use operant conditioning with magnetic stimuli have also been unsuccessful²⁵ but largely unpublished. Previous laboratory testing has therefore failed to provide a conclusive demonstration of magnetic sensitivity in the homing pigeon, leaving the suggestive results of outdoor test releases unconfirmed.

Earlier laboratory experiments, particularly those using classical conditioning, required that the pigeons be restrained from active movement. The design and construction of a flight tunnel (Fig. 1) permitted greater activity, including voluntary flight, during the testing period.

Training was undertaken to determine if pigeons could learn to discriminate between a 0.5 G induced field and the 0.02 G background field. During a preliminary period of adaptation to the testing apparatus, it was found that mated pairs of birds were much more active within the tunnel than single birds. Therefore, intensive training was undertaken with three mated pairs borrowed from several lofts.

After 2 weeks, the birds' behaviour had been sufficiently shaped to allow the recording of data. Each trial began by uniformly releasing a pair of birds at the tunnel entrance. The pigeons then travelled the length of the tunnel and were trained to enter the correct feeding box associated with each magnetic field condition. The sequence of magnet coil activation was determined at random and set before the start of each trial. To serve as a positive reinforcement, food was available only from the correct feeding box. Incorrect responses were punished by confining the bird within the box for 30 s before allowing it to enter the correct box. All trials ended with the recovery of both birds from the correct box. Only the first response of the first bird was recorded, as the second bird did not respond independently. For the duration of testing, the birds were housed in cages within a naturally-lighted and ventilated room, and maintained within 10% of their *ad lib.* weight.

Two basic patterns of response were observed during adaptation and training of the pigeons. After landing, the birds would either walk the length of the tunnel or engage in a period of spontaneous 'flutter' activity before entering a feeding box. Flutter consisted of sustained hovering, jumping, rapid turning and short flights. Trials with at least 3 s of continuous flutter activity were recorded as 'with flutter', the remainder were designated 'no flutter'. A summary of the data is presented in Table 1. The cumulative performance record for one pair of birds is detailed in Fig. 2.

The data indicate that a statistically significant discrimination had occurred. This was even more apparent when the trials were separated according to flutter activity.

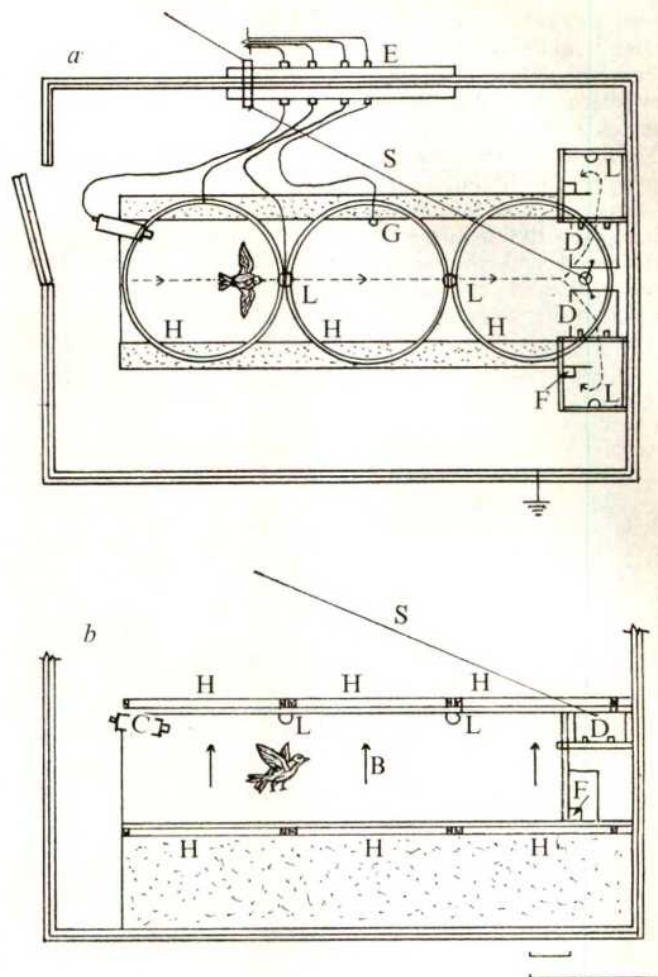


Fig. 1 Pigeon flight tunnel. Top view (a) and side view (b) of flight tunnel as constructed within mu-metal shielded room. Shielding reduced the natural magnetic field to a level of 0.02 ± 0.01 G. Three pairs of Helmholtz coils (H) were connected in series and arranged to induce a vertical magnetic field (B) along the length of the tunnel in as uniform a manner as possible. Each coil was wound with 150 turns of 12 gauge copper wire. Approximately 7.5 V d.c. at 100 mA was required to induce a 0.5 G (Earth-strength) magnetic field, as measured from a central probe (G). When the coils were not in use, the current was diverted across an external equivalent resistance. Two feeding boxes with concealed food bins (F) were located at the far end of the tunnel. For each trial, food in one bin was accessible while food in the opposite bin was covered by an aluminium screen that prevented access. Birds could be confined within the boxes by remote-controlled doors (D) which were released by string (S). Illumination was provided by 12 V d.c. lamps (L). A closed-circuit television camera (C) and electrical patchboard (E) allowed monitoring and control to proceed from a separate room which housed the power supplies, gaussmeter and television monitor. Scales: upper, 1 ft; lower, 1 m.

Table 1 Summary of flight tunnel training results

Pigeons		No. of trials	No. correct	Percentage correct	Probability*
Cornell pair	Total	150	101	67.3	< 0.0001
	With flutter	89	68	76.4	< 0.0001
	No flutter	61	33	54.1	NS
Richards pair	Total	129	89	69.0	< 0.0001
	With flutter	61	50	82.0	< 0.0001
	No flutter	68	39	57.4	NS
Walcott pair	Total	36	23	63.9	< 0.15
	With flutter	16	13	81.2	< 0.025
	No flutter	20	10	50.0	NS

*Two-tailed binomial probability for observed distribution.
NS, Non-significant (random) distribution.

In all cases, the 'with flutter' trials were statistically non-random while the 'no flutter' trials were random. Performance of the birds was not below the chance level in any category. The only factor altered from one trial to the next was the applied magnetic field. Although attempts were made to prevent any inadvertent cues (such as power supply hum), the influence of any such cues on discrimination would probably be independent of flutter activity. Therefore, the finding of significant differences between trials with and without flutter suggests that inadvertent cues or experimenter-induced bias did not influence the results.

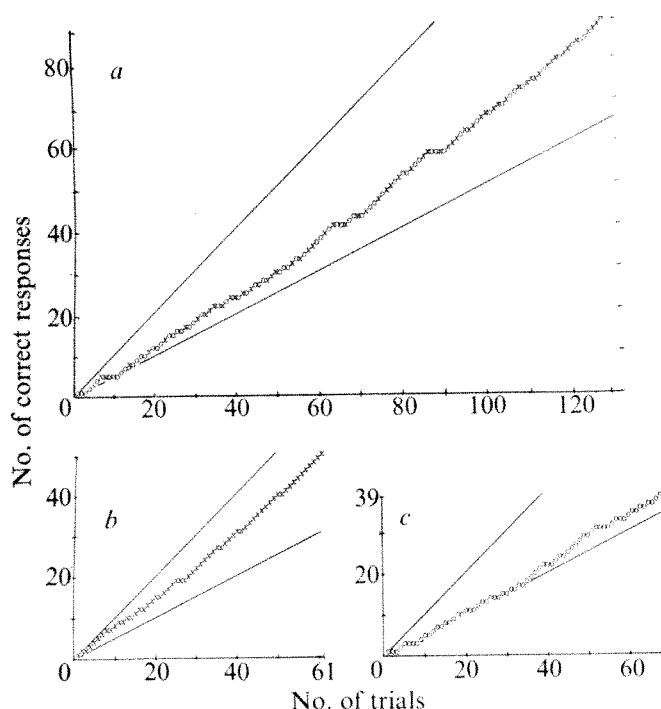


Fig. 2 Richards birds: cumulative record. Graphs for one pair of birds showing the number of trials with correct responses against the total number of trials. Only the response of the first bird to make a response was recorded for each trial. Lines indicating the 50% (chance) and 100% (perfect; upper line) levels of performance have been added. *a*, The total trial sequence is shown. Note that a significant discrimination had occurred and that 'no flutter' (○) and 'with flutter' (×) trials were distributed throughout the testing period ($P < 0.0001$). *b*, 'With flutter' (×) trials graphed separately ($P < 0.0001$). *c*, 'No flutter' (○) trials graphed separately (non-significant). Significant discrimination only occurred in the 'with flutter' trial sequence.

Thus, it seems that homing pigeons have a magnetic field sensitivity that can be demonstrated in the appropriate laboratory setting. Unlike other more conventional senses, the magnetic sense is somehow associated with flutter activity. Fluttering is also seen in successful experiments with caged migratory birds¹⁷⁻¹⁹. Although fluttering may be directly involved with the mechanisms of perception, it is just as likely to reflect a more general sensory activation that accompanies orientation. The biological mechanism of magnetic sensitivity in birds remains unknown, but may include paramagnetic, inductive or ferromagnetic interactions. The testing of pairs of birds in a flight tunnel is a straight-forward laboratory technique that could be applied to these and other unanswered questions in orientation research.

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Autogeny in saltmarsh mosquitoes induced by a substance from the male accessory gland

OVARIAN maturation by mosquitoes and other haematophagous Diptera without a prior blood meal is called autogeny, while the term anaautogeny applies to those cases where blood is necessary. Many populations of the saltmarsh mosquito, *Aedes taeniorhynchus*, contain both autogenous and anaautogenous females¹⁻². Moreover, within this species there are two types of autogenous females: (1) those that need a stimulus from mating to produce eggs autogenously and (2) those that lack this requirement³. We report that autogenous egg production associated with mating is triggered by a substance from the male accessory gland. The stimulation of normal oviposition behaviour and the termination of sexual receptivity in the female mosquito are also caused by a male accessory gland substance which is introduced into the female during mating^{4,5}. Both of these responses are uniformly exhibited by all females of several species⁶.

To demonstrate the influence of the accessory gland on ovarian maturation, we injected non-blood-fed, virgin females with tissue and fluid obtained from sonicated male accessory glands. This suspension was prepared by placing the glands of 250 virgin males in 1 ml of saline solution⁷. Since each female was injected with 1 μ l of suspension, our standard dose was equivalent to one-fourth of a pair of male glands. Males were at least 5 d old before paired accessory glands were removed. We used two closely related species, *A. taeniorhynchus* and *A. sollicitans*, both as the recipients and the sources of glands. All experiments with *A. taeniorhynchus* involved either the Rutgers stock or the Lake Charles strains. There are no laboratory colonies of *A. sollicitans*. Thus, we used F₁ offspring of wild adults collected in Indian River County, Florida. Procedures for rearing and maintaining mosquitoes followed those of O'Meara and Evans⁸.

Only a few non-injected and saline injected females developed eggs (Table 1), but over half of the *A. taeniorhynchus* injected with male accessory gland fluid (MAGF)

produced eggs in the absence of a blood meal. The ovarian stimulant associated with the gland seemed to be non-specific since *A. taeniorhynchus* responded in a similar fashion to material from either *A. sollicitans* or *A. taeniorhynchus*. The observed frequencies of autogeny in both the Rutgers stock and the Lake Charles females injected with accessory gland material were equivalent to the levels obtained in our previous studies where the females were allowed to mate³. During all treatments the vast majority of the *A. sollicitans* females remained anautogenous (Table 1). Of the 1,005 *A. sollicitans* females examined only four were autogenous. The rare occurrence of autogeny in *A. sollicitans* evidently is independent of MAGF and probably any other factor associated with mating.

The ovarian maturation associated with MAGF injections was not an artifact caused by just a general increase in the total protein concentration of the mosquito's haemolymph. Neither human serum nor protein from the fat bodies of male *A. taeniorhynchus* stimulated autogenous egg development (Table 1). For the various treatments, the amount of protein injected into each female was as follows: 10% human serum, 10.0 µg; 5% human serum, 5.0 µg; MAGF (*A. taeniorhynchus*), 0.25 µg; male fat bodies, 0.25 µg. The amount of protein that the females obtained from MAGF was quite small. Thus, most of the protein for autogenous egg production must have come from the female's internal reserves which were accumulated during the larval stage.

The ability of male accessory glands to induce ovarian development is acquired sometime after adult emergence. Females show no ovarian response when they are injected with MAGF from very young males (<1 h old).

At least three hormones are involved in the overall regulation of ovarian development in mosquitoes^{9,10}. Both autogenous and anautogenous females seem to use the same general endocrine pathway. Differences have been discovered in the mechanism controlling release of egg development neurosecretory hormone (EDNH) (ref. 11), however. Some *A. taeniorhynchus* females need no external stimulus

to trigger the release of EDNH and thus they begin autogenous ovarian maturation even before they are mated. Blood feeding will also trigger the release of EDNH and, possibly, MAGF does the same.

The association of MAGF with autogeny might represent another matrone-induced phenomenon. Matrone, a male accessory gland substance, is responsible for stimulating normal ovipositional behaviour and for terminating sexual receptivity in the female^{12,13}. These responses to matrone, however, are normally exhibited by all females in a mosquito population, whereas only a portion of the *A. taeniorhynchus* females in a population respond to the male accessory gland factor by producing eggs.

In *A. taeniorhynchus*, geographical variation in autogenous fecundity seems to be controlled by polygenic factors¹. Results of preliminary crossing and selection experiments indicate that other genetic factors control the occurrence of the various types of autogenous and anautogenous females. Attempts to identify the major genetic components involved in the regulation of autogeny are in progress.

The occurrence of two types of autogenous females within a mosquito population has been observed in other species besides *A. taeniorhynchus*. Working with the arctic species, *Aedes impiger* and *Aedes nigripes*, Corbet¹⁴ found both obligate and facultative autogeny. Females with the facultative type of development were potentially anautogenous for several days after emergence. But, if they did not succeed in obtaining a blood meal, then they switched to an autogenous mode of egg maturation. Corbet¹⁴ postulated that this change in the female's reproductive strategy was a response to the female's energy reserves, although no experimental study has produced evidence to support this theory.

Clearly, male-induced ovarian maturation represents a facultative form of autogeny and the switch from potentially anautogenous to autogenous development is mediated by a chemical from the male accessory gland. Most *A. taeniorhynchus* females are refractory to insemination for at least 36 h after emergence^{15,16}. The average duration of this refractory period is about 72 h in both the Lake Charles and Rutgers stock strains. Where insemination is a prerequisite for autogeny, the refractory period would contribute to the delay in the onset of ovarian maturation. As long as the ovaries remain undeveloped the females continue to be active blood-feeders and the fecundity in the initial egg batch is greatly enhanced in those mosquitoes that feed at this stage. Such a flexible system involving autogeny enables the females to capitalise on the availability of suitable hosts while at the same time it provides a means whereby egg maturation can occur in the absence of any host. Additional studies are needed to determine if mating and MAGF influence ovarian maturation in other mosquito species.

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Table 1 Effect of injected male accessory gland fluid (MAGF) from *A. taeniorhynchus* (AT) and *A. sollicitans* (AS) on females of each species

Treatment*	Females		Eggs per female†
	No. examined	% Autogenous	
<i>A. taeniorhynchus</i> (Lake Charles)			
MAGF-AT	556	53.1	22.1
MAGF-AS	218	62.8	21.5
Saline	484	4.5	20.8
Non-injected	615	2.6	25.4
<i>A. taeniorhynchus</i> (Rutgers stock)			
MAGF-AT	442	55.9	18.7
MAGF-AS	139	66.9	17.4
Saline	583	3.9	18.1
Human serum (10%)	73	1.4	15.0
Human serum (5%)	72	0.0	—
Fat bodies‡	72	1.4	11.0
Non-injected	950	1.9	19.2
<i>A. sollicitans</i>			
MAGF-AT	208	0.0	—
MAGF-AS	274	0.4	15
Saline	293	0.7	10
Non-injected	230	0.4	31

Sexes were separated within 3 h after adult emergence. All tests were conducted with virgins who had access to 10% sugar solutions. Females were denied oviposition sites and they were considered autogenous only when at least one ovarian follicle had completed maturation to stage V. Males were at least 5 d old before their accessory glands were removed. If mated the autogeny rates were about 50% in both the Rutgers stock and the Lake Charles strains (see ref. 3).

*Females injected with 1 µl of solution 2–3 days after emergence and then examined for ovarian development 7 days later.

†Mean no. of eggs per autogenous female.

‡Fat bodies from the abdomens of male *A. taeniorhynchus*.

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Influence of the seminal plug on mating behaviour in the garter snake

PHEROMONES are used by a wide variety of animals to identify the social status, sex and reproductive state of conspecifics¹. In snakes, olfactory cues are involved in prey selection and trail following^{2–4}, recent experiments indicate that female sexual attractivity in the garter snake *Thamnophis sirtalis* is oestrogen-dependent and that sexually active males discriminate between, and preferentially court, oestrogen-injected females^{5,6}. We report here that in the closely related species, *T. radix*, sexually active males do not court recently mated females. This discrimination is based on the presence of a seminal plug normally deposited by the mating male during copulation, and the mating plug exerts an inhibitory effect on the courtship activity of other males.

Courtship and copulation in *Thamnophis* are readily identifiable behaviours. Male approach is visually guided^{7,8} and courtship behaviour is mediated by tongue-flick delivery of odour molecules to the vomeronasal organs as in trail-following^{2,3,7,9}. The male orients to and approaches the female, tongue flicking rapidly^{5–7}. At first contact, the male frequently drapes his tongue over the female's back, the probable source of the sex attractant⁷. If the female is sexually attractive, the male will begin to 'chin rub', a behaviour in which the male places his chin on the female's back and repeatedly traverses her length. The male then aligns his body beside the female's with his head resting behind the female's. Palpations with the posterior third of the body follow as the male slides back to oppose the cloacae. Female sexual receptivity is characterised by lifting the tail and gaping the cloacae¹⁰, stimulating the male to evert and intromit a single hemipenis. Snake hemipenes are complex structures with numerous calyces and spines, believed to have a role in the copulatory lock¹¹. Copulation in the laboratory has an average duration of 91 min (range: 28.22–188.73 min) and is very vigorous. Sperm transfer is followed by the deposition of a gelatinous material produced by the renal sex segment, forming a copulatory plug^{8,12,13}; it has been suggested that the plug functions to reduce sperm leakage and prevent insemination by other males (ref. 13, but see ref. 14). Following separation, the male is sexually refractory for at least 24 h.

To assess the effect of mating on female sexual attractivity, attractive females were retested for sexual attractivity after having been allowed to mate. Female attractivity decreased markedly after mating and the decline was immediate and long-lasting (Table 1). Most non-mated males failed to court mated females even though they had courted the same female immediately prior to the female's copulation.

To determine the role of the mating plug in the post-mating decline in female attractivity, the mating plug was expressed and the female's cloaca laved with 10 ml distilled water immediately following mating. Females were then retested for attractivity (see Table 1 for protocol). Females with mating plugs removed remained attractive to sexually active males: seven of eight females were courted during the retest.

As in lizards and various other vertebrates and invertebrates (reviewed in ref. 15) mating in *Thamnophis* seems to inhibit further female sexual receptivity. In lizards, this decline in receptivity following mating is immediate and a consequence of male intromission¹⁵. In the present experiment, recently mated females which had had the mating plug removed were courted vigorously but failed to raise

the tail and gape the cloaca in response to male palpation for at least 48 h after mating; thus, they were attractive but not receptive.

To establish further that the post-mating decline in female attractivity was related to the mating plug, a third experiment was conducted in which cloacal material from both recently mated females and from courted but unmated females was collected and applied to sexually attractive females. Results demonstrated clearly that the mating plug is responsible for the loss of female attractivity following mating (Table 2). Females rubbed with cloacal material from unmated females or distilled water were actively courted; however, most females with cloacal material from recently mated females were not courted. Most females were courted after the applied material was removed with a wash of alcohol followed by water.

As these experiments were in progress, it became apparent that the behaviour of sexually active males changed dramatically following exposure to a mated female. To assess the effect of exposure to a mated female on subsequent male courtship, recently mated females were introduced into cages containing proven sexually active males (as determined by consecutive male courtship of two injected but unmated females immediately before presentation of the mated female). After a 5-min test with a mated female, the unmated females were reintroduced in order and male courtship of each scored. Exposure to a mated female precipitated a marked reduction in male courtship activity; if exposed to a mated female, previously sexually active males (nine of eleven) did not court females they had just courted. The duration of this effect varied between males.

T. radix exhibit a reproductive cycle typical of high latitude species. In the spring, breeding is restricted and compressed into the first days after emergence from the winter hibernaculum^{12,16}. Males emerge several days before females¹⁷ but do not leave the area. On emergence, females are courted *en masse* by the males in the immediate vicinity; mating 'balls' of 100 or more males courting a single female have been reported. It is not known if this mass courtship facilitates female receptivity. It appears that once a single male intromits, the unsuccessful males abruptly leave the copulating pair^{7,12,18}, further, recently mated females are usually not courted¹².

Table 1 Influence of mating on female attractivity in the garter snake *Thamnophis radix**

Treatment	N	No. of females courted
Pre-mating	17	17
Post-mating:		
5–20 min	9	0
24 h	16	2†
36–48 h	11	3†

*Not all females were retested for attractivity at all intervals post-mating. All behavioural tests were 5 min in duration. Males were considered to be sexually active if they courted the female uninterrupted for 30 s; a female courted consecutively by two males was considered sexually attractive. Precautions were taken to avoid transferring odours through handling. Animals were purchased in September from a commercial supplier in Wisconsin. Snakes were handled 1–2 ml of a meat-egg-vitamin mixture each week. Water for drinking and soaking was available *ad libitum*. Male snakes were kept individually in wire mesh enclosures (61 × 22 × 77 cm). Unmated females were housed in groups of six in wire cages; mated females were separated and caged individually. Cages containing males and testing cages were inside Sherer Gillett RI-48-LLTP environmental chambers programmed to provide a 14:10 L:D photic cycle with a corresponding thermal regimen of 32:23 °C; females were exposed to a 14:10, 27 °C daily photothermal cycle. All snakes were intact and received the following hormone treatment. Males were induced to a sexually active state by subcutaneous (s.c.) implantation of a 2.0 cm silastic capsule (0.15 mm inside diameter) containing approximately 16 mg of free testosterone. Females received daily s.c. injections of oestradiol benzoate^{5,6} (50 µg per day); injections were discontinued on the eighth day. N, no. of animals tested.

†In two cases, post-mating courtship involved the same test male.

Table 2 Role of mating plug in mating-induced decline in female attractivity

Treatment	N	No. of females courted
Pre-test:		
Untreated	18	18
Test:		
Cloacal wash		
mated female	7	1*
unmated female	7	7
Distilled water	4	4
Post-test:		
Alcohol-water wash	18	15†

Females were tested for sexual attractivity using protocol described in Table 1. In the first of three consecutive courtship tests, the sexual attractivity of the females was established. Cloacal material from recently mated females or unmated females, or distilled water, was then applied to the dorsal surface and the females were retested for attractivity. Females were then washed with alcohol (90%) followed by distilled water to remove the applied substance before being retested for attractivity. N, no. of animals tested.

*Male courtship weak.

†Of the three non-courtied females, two were not courtied during the post-test period because of the decline in male courtship behaviour as a result of the exposure to mating plug material (see text).

These field observations of male dispersal from the mating pair suggest that the mating male deposits an odour(s) during copulation which communicates to other males the mated status, and hence non-receptivity, of the female. Thus, it is possible that the mating plug may, in addition to aiding in sperm retention, enable other sexually active males to determine whether a particular female has mated recently.

The experimental finding that exposure to odour(s) secreted during copulation (presumably by the male's urogenital system) results in sexually active males 'losing interest' in unmated, sexually attractive females, however, suggests an alternate explanation for male dispersal from the mating ball. In those insects having dense mating aggregations in which the probability of multiple inseminations is high (intense sperm competition), mechanisms have evolved which reduce the likelihood of multiple inseminations¹⁹. It is possible that, in *Thamnophis*, the odour(s) deposited by the mating male maximise the male's reproductive success by subsequently decreasing female attractivity as well as temporarily removing other males from the breeding population (that is, while the recently mated male is sexually refractory and unable to mate). Male dispersal from the mating ball, therefore, could be a consequence of unsuccessful males avoiding these odour(s). The demonstration and clarification of either or both of these postcopulatory adaptations must await detailed field studies of *Thamnophis* sexual behaviour.

Field observations also indicate that females leave the vicinity of the hibernaculum soon after emergence and copulation¹⁷. Since copulation is prolonged and conspicuous in *Thamnophis*, the mating pair is presumably very vulnerable to predation at this time. Mating-inhibition of further female sexual attractivity and receptivity, therefore, may act along with the female's departure from the hibernaculum area to minimise the female's vulnerability to predation (see also refs 15, 17).

Although our findings may be due to the restricted testing conditions, they demonstrate that sexually active males do not court sexually attractive females after prolonged or forced exposure to copulation odour(s). The specificity of this decline in male courtship behaviour as a consequence of exposure to a mated female is indicated by the observation that such males continue to investigate introduced snakes and trail-follow. It would thus be of interest to know whether this cessation of courting behaviour is due to a change in sensitivity at the peripheral or central level. In any case, the demonstration in verte-

brates that the mating plug of one male can influence the behaviour of other males toward other females is, to the best of our knowledge, unique.

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Copulatory plugs, restricted mating opportunities and reproductive competition among male garter snakes

REPRODUCTIVE competition for females among males of a given species can take many forms, some of which (like mating plugs) may place constraints on the mating opportunities of males and intensify competition for available females. I have studied the reproductive behaviour of two garter snake species (*Thamnophis sirtalis* and *T. butleri*) in the field for 5 years. They do not exhibit pair-bonding, paternal care of offspring, territoriality, or intrasexual combat. Sexual selection in this mating system should favour males which compete to locate, court and mate with the maximum number of females¹. Yet I have observed numerous solitary female *Thamnophis* within the same time periods and areas in which other females were being vigorously courted, often by several males. I have previously observed that the anterior cloaca of any recently mated female *Thamnophis* contains a copulatory plug which occludes the oviductal orifices². The plug evidently is formed by the copulating male after sperm transfer. I interpreted this as a form of intrasexual competition in which the successfully copulating male makes the female temporarily unavailable to other males and reduces the likelihood of multiple inseminations. Some reports^{3,4} indicate that multiple inseminations are possible. I present here evidence from the field that male garter snakes recognise females with a copulatory plug and behave as if these females were unavailable.

The pattern of emergence from hibernation and mating has been described for *T. sirtalis* in Canada⁵⁻⁸, but my own observations differ in several important respects. Mating activity in southeastern Michigan is most intense on warm days in March and April immediately following the emergence of these snakes from hibernation, but before they disperse from the denning area. A denning area in Michigan is a collection of separate refuges, such as crayfish or rodent burrows, no one of which is used by all of the snakes at

that area. Males are conspicuous throughout the mating season, either courting or actively moving through the denning area evidently searching for females which might be emerging from any of the refuges. The emergence of females from hibernation is sporadic with individuals appearing on different dates. Females showing evidence (the copulatory plug) of recent mating do not immediately disperse from the denning area. These females, however, were never being courted when I encountered them. By contrast, those females which did not have a plug were usually being courted (Table 1).

The hypothesis that females with cloacal plugs are unattractive to males was tested in the field. Individual male snakes were given the opportunity to court first a female with a plug and then a female without a plug. The results (Table 2) clearly show that sexually active males can recognise and are disinclined to court females with plugs. All of these males subsequently courted the females without plugs.

Table 1 Observed mating status and courting activity of female garter snakes

	No. being courted	No. not being courted	χ^2
<i>T. sirtalis</i>			
without plug	21	6	27.2
with plug	0	19	
<i>T. butleri</i>			
without plug	14	3	9.9
with plug	0	4	

Values are the total number of females observed at the mating areas during the mating seasons from 1974 to 1976. Mating season is defined for each year as the time from the first observed courtship to the last.

A male can increase his opportunities for mating by minimising the time spent in unproductive courtship. It would be advantageous for a male to recognise and disregard females with plugs if plugs effectively prevent further inseminations. Among males in competition to locate unmated females, those that can recognise the altered mating status of a female as soon as possible should be favoured. Frequently, several males court a female simultaneously. When one mates, the unsuccessful males tend to disperse, presumably seeking other females, before their successful rival even finishes mating. I have seen this occur three times in the field and similar reports from the field⁹ and the laboratory^{10,11} exist. Even the successful male should soon be seeking other females. In at least four cases, males that I had observed mating were courting another female within

Table 2 Responses of male garter snakes to females with or without copulatory plugs

Species	N	Courted both	Courted neither	Courted only female with plug	Courted only female without plug	Probability
<i>T. sirtalis</i>	11	2	0	0	9	<0.01
<i>T. butleri</i>	13	0	0	0	13	<0.001

The mid-body portion of a female with a plug was carefully set in front of each male. Both the head and anal portions of the female were held by hand to prevent her retreat into the undergrowth. If the male contacted the female with his tongue and initiated normal courtship by aligning his body along the coils of the female's body with his chin pressed tightly against her dorsal skin, his response was scored as positive. A response was scored as negative only after a male contacted the female with his tongue several times and then ignored her. As soon as a score was obtained for a female with plug, she was removed and the procedure was repeated on the same male using a female without a plug. In this matched-pairs design, each male served as his own control. Probabilities are for a one-tailed sign test.

an hour. More extensive data on males mating more than once in one afternoon have been reported¹².

The mechanism by which male garter snakes recognise the altered mating status of females with plugs remains unknown. In my experimental field trials, the males did not seem to behave differently toward either female until tongue contact was made. The males made no attempt to investigate the female's cloacal area (which was within my hand) before courting or rejecting her. This suggests that a chemical cue which is not physically associated with the plug itself may be involved. I doubt that the cue could be from the plug itself inasmuch as that is a male product. If a male were capable of producing any substance which directly caused other males to disregard a female, it would be most advantageous to use it at the beginning of courtship to avoid competition from courting rivals, rather than reserving it for use (in plugs) after mating was completed. This is not observed. If this potential advantage ever existed for some males, competing males that disregarded male odours and courted anyway would be favoured. Alternatively, plugged females could find it advantageous to 'announce' their altered status, since courting aggregations may be just as conspicuous to potential predators as they are to human observers. If fruitful mating is unlikely, females should not risk predation. Therefore, the female is probably responsible for producing the cue.

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Chameleons use accommodation cues to judge distance

Any animal that adjusts the focus of its eye lens has monocular distance information potentially available from its focusing mechanism. Such accommodation cues are independent of size and context, and thus could provide an objective measure of distance. Ingle has proposed that such information is used by monocular frogs¹ and toads², both of which he has shown to be capable of estimating distance with one eye³. There is no direct evidence, however, that any animal perceives distance as a function of the plane of focus of the image. The experiments described here demonstrate that chameleons do so in estimating the distance of their prey.

It has not been shown that chameleons judge distance when feeding; however, their method of catching insects seems to be sufficiently exacting to require accurate distance assessment: the tongue is shot out with remarkable speed ($\sim 5 \text{ ms}^{-1}$) to hit and retrieve insects at distances up to 1.5 times body length⁴ (Fig. 1). The eyes of a chameleon normally move independently, each eye scanning (saccadically) over more than a hemisphere. While taking aim at an insect, however, both eyes are swivelled directly towards the target (Fig. 2), often prompting the assumption that these animals acquire distance information by some process of triangulation.

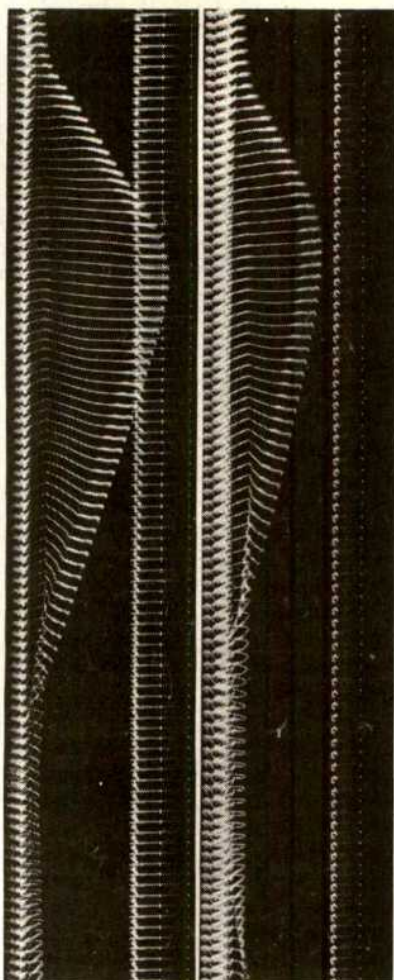


Fig. 1 Multiple exposure photographs of a chameleon attempting to capture an insect. 35-mm black and white film (Kodak Tri-X) was loaded into an oscilloscope camera, and the shutter left open. Illumination was provided by a strobe light flashing at 2.5 ms intervals. The camera motor drive was triggered by hand as the chameleon took aim and the film then moved past the shutter at 16 inches s^{-1} , thus separating the frames. The chameleon is on the left and is viewed from the side; only his head is visible. The insect he is attempting to catch is on the right, mounted on the end of a wooden stick (hence the series of horizontal bars on the right). The scale is given by the marks on the stick (5-cm spacing). Time moves down the page. At the top the chameleon's tongue can be seen protruding from its mouth as it takes aim. Then the tongue can be seen shooting across the gap towards the insect. *a*, The chameleon is wearing lateral prism spectacles (see Figs 2 and 3) and the tongue passes freely by the insect, before being retracted. *b*, In contrast, when the animal is wearing negative lens spectacles (see Fig. 3), the tongue never reaches the insect. This undershoot is predicted from the hypothesis; but naturally occurring undershoots in healthy adult chameleons are extremely rare (less than 1 per 250 attempts).

Chameleons were filmed while attempting to catch insects at various distances. The species used was *Chameleo jacksoni*, which has horns from which opaque screens, lenses and prisms could be suspended such that the animal was required to look through them as it took aim (see Fig. 2). This made it possible to manipulate what the chameleon was seeing while it was taking aim.

If chameleons judge prey distance, then one might expect some quantitative aspect of their behaviour to correlate with the range of the insect (the object distance, D_{obj}). Occasionally, a chameleon will miss the target, and it is then possible to measure the maximum distance the tongue reaches (maximum tongue extension, S_{max}). This unobstructed tongue flight can be produced consistently by providing the animal with an illusory target (Fig. 3*a*). The results of a series of such binocular shots are shown

in Fig. 3*b*. There is a positive correlation between the behavioural measure (S_{max}) and the distance of the insect (D_{obj}). A similar series was filmed of animals feeding monocularly, and a similar correlation was found to exist (Fig. 3*c*). Thus chameleons do judge prey distance both monocularly and binocularly, as in both cases they modify their behaviour according to the target distance.

Chameleons have a focusing mechanism³, so in theory they could use accommodation cues to estimate distance monocularly (or binocularly). When the ciliary muscles of the eye are at rest, parallel rays of light are brought into focus on the retina⁴. As an object is brought towards the eye, however, at some critical distance the image will no longer be in focus, and the lens must subsequently be deformed continuously to maintain a clear retinal image as the object moves closer. Within this accommodating range, the degree of lens deformation, or the motor command used to effect that deformation, could give a measure of the distance of the object fixated.

It is possible to test whether chameleons use accommodation cues while feeding by altering the plane of focus of the prey. For instance, the image of an insect viewed through a biconcave (negative) lens lies closer to the animal than the insect itself. Thus if an animal estimates distance as some function of the plane of focus, it would undershoot in this situation. Conversely, it would overshoot if wearing biconvex (positive) lenses. For the animals for whom control data exist, it is possible to calculate quantitatively their predicted behaviour wearing spectacles with lenses of any strength. The curves drawn above and below the regression lines in Figs 3*b* and 3*c* show the expected deviations from normal behaviour for the particular focal length lenses used in the experiments. There is a close fit between the data obtained experimentally and the behaviour predicted under the hypothesis that chameleons estimate prey distance as a function of the plane of focus.

None of the other recognised cues to distance gives

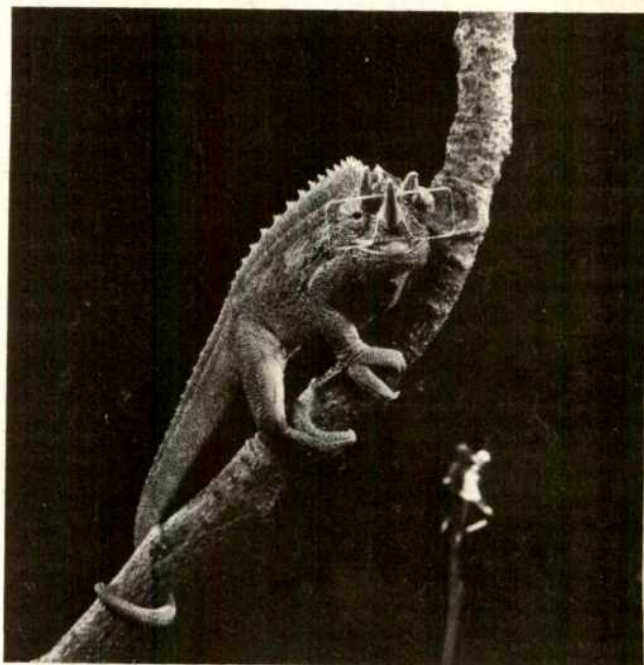


Fig. 2 A chameleon wearing spectacles. Chameleon eyes normally move independently, each eye scanning (saccadically) over more than a hemisphere. When taking aim at an insect, however, both eyes are swivelled directly towards the target, and chameleons always aim and shoot their tongues straight ahead. Thus spectacles can be positioned so that the animal must look through them while taking aim.

predictions at all comparable with those from the accommodation theory. Indeed, of the monocular cues, motion parallax, retinal image size and context cues such as texture gradients and perspective, all change in such a way that the predicted behaviour for an animal wearing lenses would be qualitatively the opposite of that actually observed. Neither of the binocular cues, retinal image disparity or the convergence angle of the eyes, is in theory altered by the spectacle lenses, although it could be argued that in practice the animal will probably not be looking through the optical centres of the lenses, so there will be prismatic effects that may alter the convergence of the eyes. Chameleons, however, seem unaffected by alteration of the binocular convergence angle (Fig. 3b).

In conclusion, binocular and monocular chameleons

perceive prey distance as some function of the plane of focus, and accommodation is not just used as a back-up cue when binocular information is unavailable.

If an animal uses its focusing mechanism as its primary source of distance information, one might expect the structure of the eye to have design features maximising the accuracy of such a cue. To maximise the detection of error in focusing, the eye should have good angular resolution; the organisation of the chameleon eye suggests that these animals, particularly for their size, have high acuity vision. Chameleons⁵ have a well developed fovea, comparable in general appearance (deep convexiculate) with that of birds of prey^{3,7} renowned for their visual capabilities. Rochon-Duvigneaud's⁸ estimate of the chameleon foveal receptor cell density is probably much too high

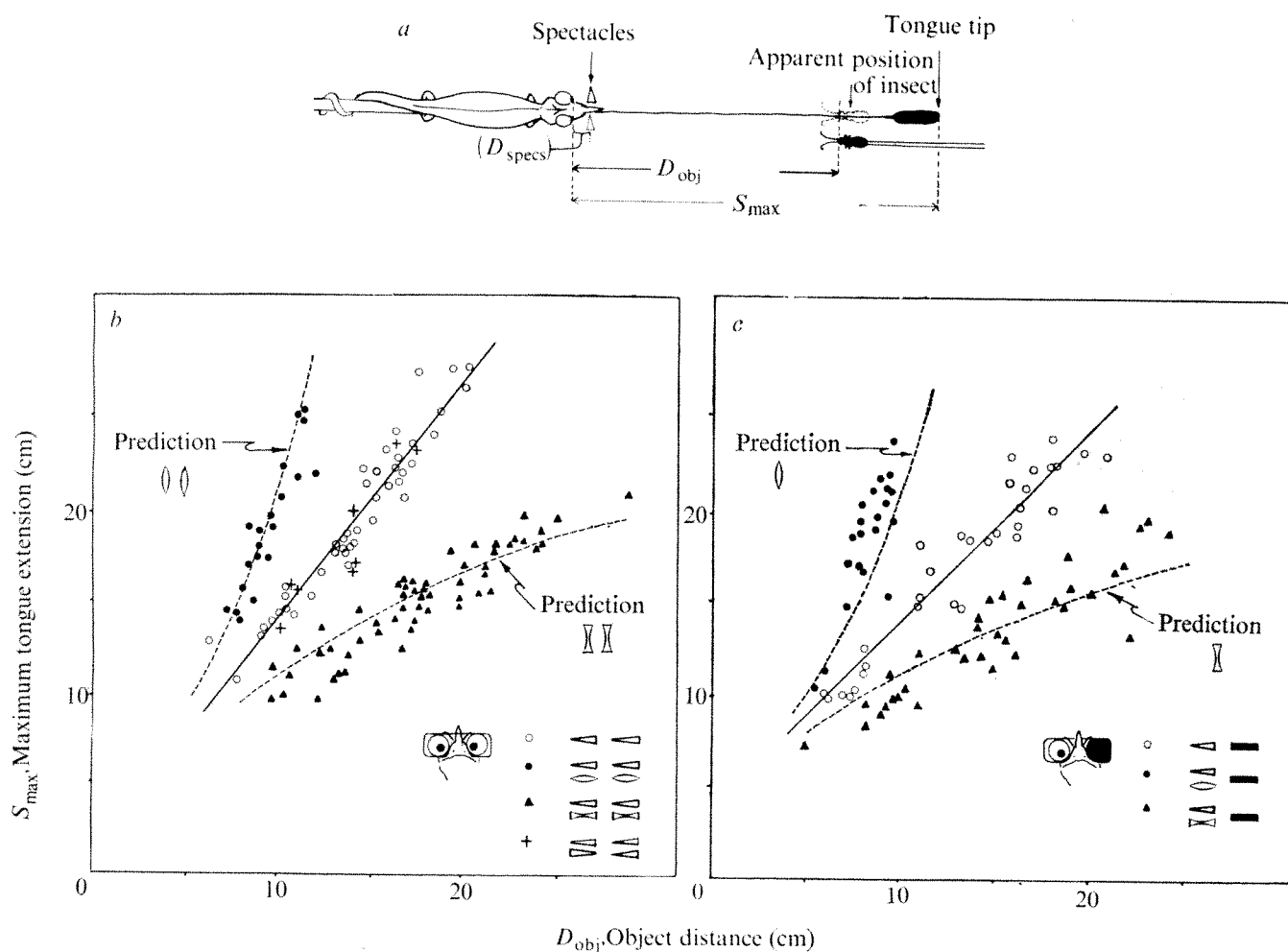


Fig. 3 *a*, A chameleon viewed from above while shooting at an illusory target. The image of the insect is displaced laterally by thin prism spectacles, and the tongue in consequence passes by to the side of the real insect. The object distance (D_{obj}) is defined as the distance between the near side of the insect and the chameleon's pupils, measured on the film frame just before the tongue is fired. The maximum tongue extension (S_{max}) is defined as the distance between the pupils and the tongue tip, measured on the film frame where the tongue is maximally extended. *b*, *c*, The relationship between the range of the insect target and the maximum tongue extension for binocular (*b*) and monocular (*c*) animals wearing spectacles (shown in section on the figures). Each point represents one attempt by one of the three animals tested. ○, Attempts where animals wore just lateral prism spectacles; —, regression lines fitted to the data giving equations of the form

$$S_{max} = a(D_{obj}) + b \quad (1)$$

---, Overshoots and undershoots predicted by the hypothesis for animals wearing lens spectacles. The distance of the focal plane from the chameleon's pupil ($D_{focal\ plane}$) can be calculated from the lens equation, the distance between the eyes and the spectacles (see Fig. 3a) being taken into account:

$$D_{focal\ plane} = D_{specs} + 1/[1/F - 1/(D_{obj} - D_{specs})]$$

where F is the focal length of the lens. Predicted S_{max} can then be calculated for different insect ranges, by substituting $D_{focal\ plane}$ for D_{obj} in equation (1). Using the appropriate values for a and b , curves were calculated for negative lenses (focal length -25 cm) and positive lenses (focal length $+22.3\text{ cm}$). These are the curves shown. ●, ▲, Data points obtained from animals wearing these lenses as spectacles. The close fit between the data and the predictions suggests that both monocular and binocular animals estimate prey distance as a function of the focal plane. In contrast, note the crosses (+) on Fig. 3b. These points were obtained from binocular animals wearing convergent prisms of sufficient angular deviation to predict dramatic undershoots if the animals were estimating distance as a function of the convergence angle of the eyes. Chameleons do not seem to triangulate to estimate distance.

(760,000 mm⁻²), but his ordering of chameleon cone densities (the greatest of all lizards) as lying between that of man and birds of prey, may be correct. Notice also how large the chameleon's eyes are compared with the head (Fig. 2). Absolute increase in eye size improves resolution if the receptor density remains constant, because with increasing eye size, a given object will project a larger image on the retina.

To maximise the error in focus itself, the system should have poor depth of field, which can be achieved with a wide aperture. Chameleons are capable of some pupillary constriction, yet in spite of high ambient light levels and the proximity of the insect (both of which would induce pupillary constriction in other animals⁹), chameleons maintain pupil dilatation while aiming (f /number of ~ 5). It is possible to hypothesise why chameleons should have evolved high acuity vision, for reasons other than to maximise the usefulness of accommodation cues to distance; however, the combination of good image resolution and poor depth of field is less easy to explain in other terms.

Walls⁶ expressed surprise that although lens-accommodating mechanisms are widespread within the animal kingdom, focusing mechanisms seemed to have failed to evolve into depth-sensing organs. I suggest that chameleons have evolved just such a system, and that it is possible that we may have underestimated the usefulness of accommodation cues to other animals.

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Stereopsis in toads

HARKNESS'S demonstration¹ that chameleons rely primarily upon accommodative cues to judge distance encouraged me to examine the mechanisms of depth vision in an amphibian (*Bufo marinus*). Unlike chameleons which have highly mobile eyes, toads make neither convergent nor fixating eye movements, so that, except for stabilising reflexes, their eyes are almost locked in their sockets². For simple forms of stereoscopic range finding it is an advantage to have immobile eyes because disparity measurements can be converted directly into estimates of absolute depth (Fig. 1a). One might therefore expect toads to use retinal disparity in judging the distance of their prey. Toads have the essential apparatus for stereopsis: there is a substantial binocular overlap both in front of and above the animal³, the inter-

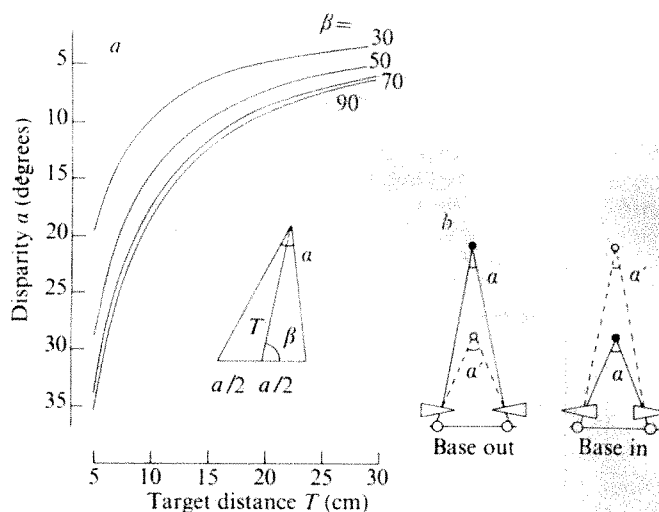


Fig. 1 a, Plot of the relation between disparity (a) and target distance (T) for a toad with an interpupillary distance of 3.2 cm. This value was typical of the toads tested. The relation between a and T depends on the lateral position of the target with respect to the toad's mid-line (β). However, this effect was negligible for the values of β that occurred in these experiments. b, Diagram to show how base-out and base-in prisms cause toads to misjudge the distance of a target. The dashed lines and open circles indicate the apparent lines of sight and positions of the target.

ocular distance is relatively large, and neural pathways bring information from corresponding areas on the two retinæ to the same region in both mid-brain⁴ and thalamic⁵ visual areas. My results suggest that toads do indeed make disparity measurements and should be added to the growing list of vertebrates known to achieve stereopsis (monkey⁶, cat⁷, owl⁸). In addition, toads have a mechanism for measuring depth within their extensive monocular field. Ingle has shown⁹ that frogs and toads, like chameleons, can estimate the distance of prey almost as well with one eye as with two, and the present data suggest that they do this by monitoring the accommodative state of their eyes when the prey is in optimum focus, as Ingle originally proposed¹⁰.

The accuracy with which toads assess the distance of their prey was determined from films of toads catching meal worms on the ground. Films were taken from above at 24 or 40 frames s⁻¹. When a toad snaps at prey within its frontal field it leans forward, extending its tongue. Two measures were derived from the films. 'Target-distance' (T) is the distance from a point midway between the eyes before the toad moves to the middle of the prey (or, when large dummy stimuli were used, a point 0.5 cm from its nearest edge). 'Snapping-distance' (S) is the distance from the same point between the eyes to the tip of the tongue at its fullest extent. Figure 2a shows that the tip of the tongue tends to overshoot the centre of the prey by about 1 cm. Thus a toad's snap includes an accurate estimate of target-distance.

The cues toads use to judge distance were studied by filming prey-capture at 40 frames s⁻¹ while toads wore lenses or prisms which distorted vision in their frontal field. Spectacle lenses alter the effective optical power of the eye so changing the normal relationship between the distance of an object and the lens position in which the image is in focus on the retina. Accordingly, a spectacle lens will systematically distort depth estimates that depend on the position of the lens. Negative spectacle lenses decrease the apparent distance of an object from u to $1/(1/u - 1/f)$, where f is the effective focal length of the spectacle lens at the principal point of the eye, and u the actual object distance. Prisms were used to assess the importance of stereopsis. Because amphibia have no fovea from which corresponding points in the two retinæ can be labelled, disparity is defined in a slightly unusual way, as the angle formed between lines connecting a target to the nodal point of

each eye (Fig. 1a). This is geometrically equivalent to the conventional meaning of disparity for conditions in which the lines of sight through the foveas are parallel. For a target that is straight ahead the relation between disparity and distance is given by the expression $\tan \alpha/2 = a/2T$, where T is the target distance and a the interpupillary distance. Prisms appropriately aligned change the apparent disparity of the target and thus alter the apparent target distance for an animal using stereopsis (Fig. 1b). Prisms that are base-out over each eye increase the apparent disparity (α') of the target by an amount equal to the summed angular deviation produced by the two prisms

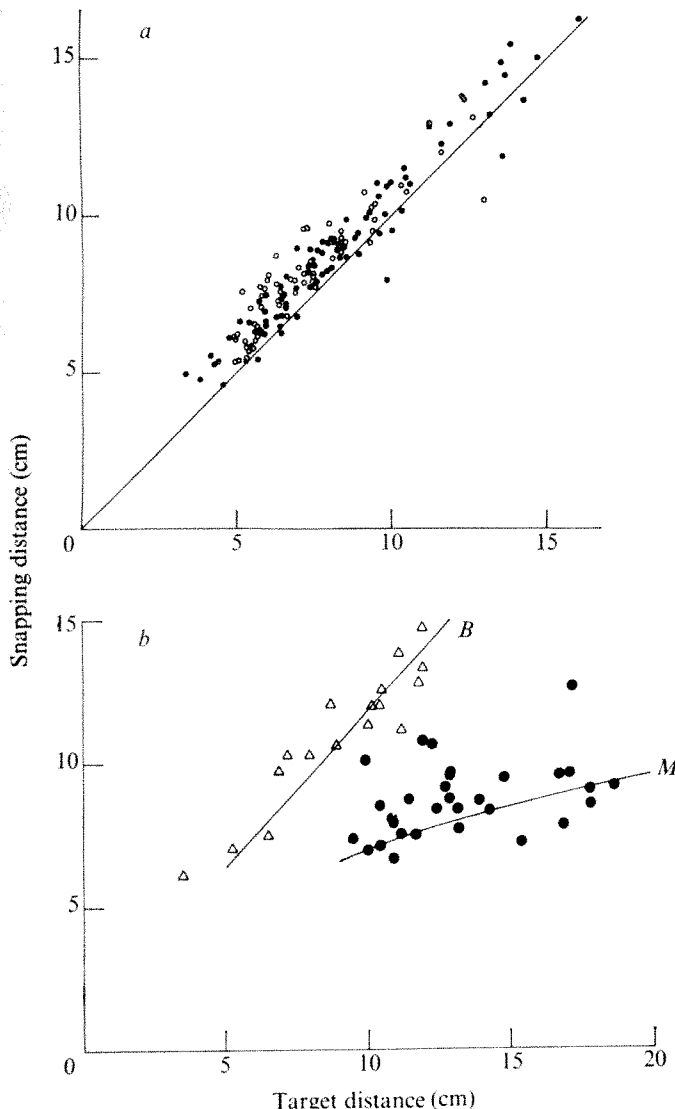


Fig. 2 *a*, Accuracy of prey capture in binocular and monocular toads snapping at meal worms moving on a dark substrate. Snapping distance is plotted against target distance. In this and subsequent graphs each data point represents a single snap. The toads tend to shoot about 1 cm beyond the centre of the target. ●, Data from seven binocular toads. ○, Monocular: data from a single toad which arrived from the animal dealer with one eye missing. *b*, The effect of -136 mm negative lenses on snapping distance. ●, Monocular: lens placed in front of one eye while the other eye is occluded. The line labelled *M* is the predicted snapping distance on the assumption that toads employ accommodative cues. △, Binocular lenses over both eyes. Line *B* is the predicted snapping distance (taking into account the prismatic effect of the lens), assuming that toads depend upon stereopsis and ignore monocular cues. When vision is monocular the toad appears to use accommodative cues, but when vision is binocular disparity measurements are more important. Data from one toad snapping at a yellow cylinder moved by hand in its frontal visual field.

at the eyes (2δ); that is $\alpha' = \alpha + 2\delta$. Base-in prisms have the opposite effect ($\alpha' = \alpha - 2\delta$). Thus, given the normal relation between S and T (Fig. 2a), the snapping distance can be predicted for a variety of lens and prism combinations. The fact that this technique can be applied successfully implies that snapping is an open-loop response in the sense that the toad decides how far it is going to extend its tongue before beginning the movement.

The importance of accommodation as a cue to distance is demonstrated by the effect of a negative lens ($f = 136$ mm) on the snapping behaviour of a toad with one eye occluded (Fig. 2b). The toad then snaps short at prey in its frontal field. The data points fall around the line labelled *M* which predicts S on the assumption that distance measurements rely solely upon accommodative cues. If motion parallax or image size were significant cues to depth for monocular toads, negative lenses would cause overshooting. Some additional evidence also indicates that these cues are not important: (1) snapping accuracy is unaffected by prey size, and (2) when a target suddenly appears in front of a monocular toad, the toad usually snaps directly at it, without making any previous head movements. (One unexplained finding is that monocular positive lenses do not cause overshooting.)

Toads have a visual field of almost 360° within which they can detect prey. If the prey is close enough, toads snap at it directly without any prior orienting movement. Thus the toad's use of accommodative cues is not limited to a restricted region of retina. It must be less sensitive than that of the chameleon which always directs its fovea at prey before shooting out its tongue, so maximising its ability to detect and minimise blur.

The performance of toads wearing negative lenses over both eyes implies that disparity is the major cue to depth when targets are viewed binocularly. The snapping data then fall around the line labelled *B* (Fig. 2b) which is the predicted snapping distance on the assumption that disparity is the only measure of target distance. Tests with prisms reinforce this conclusion. Base-out prisms over both eyes cause the toad to undershoot by an amount which is greater the larger the angular deviation of the prisms (Fig. 3a), while base-in prisms cause overshooting. Prisms which are base-in over one eye and base-out over the other do not change the disparity of the target and accordingly have no effect on snapping distance. Instead the toad misses its prey laterally. Similarly, a base-out prism over one eye and an occluder over the other leads to lateral errors but no undershooting.

Base-out prisms produce a conflict between accommodative and stereoscopic information. Snapping distance is then a compromise between these two cues, heavily weighted in favour of stereopsis. Figure 3a shows that toads shoot beyond the point predicted by the apparent disparity of the prey by an amount that increases with prism strength (that is, with the magnitude of the discrepancy between the two cues). The relative importance of monocular and binocular cues was computed from snapping data taken from binocular toads wearing base-out prisms or combinations of base-out prisms and -136 mm negative lenses (Fig. 3b). For a wide range of prism strengths the data are well fitted by the linear expression

$$S = 0.94B + 0.06M + C$$

where C is a constant of about 1 cm. The expression holds for values of M between at least 8 and 30 cm and for values of B ranging from 5 to 15 cm. Thus, when vision is binocular, disparity is the predominant depth cue and accommodative cues are very largely ignored. Nevertheless, when vision is restricted to one eye, accommodation gives adequate distance information for targets in exactly the same part of the visual field (Fig. 2a).

If stereopsis is to operate over these distances without

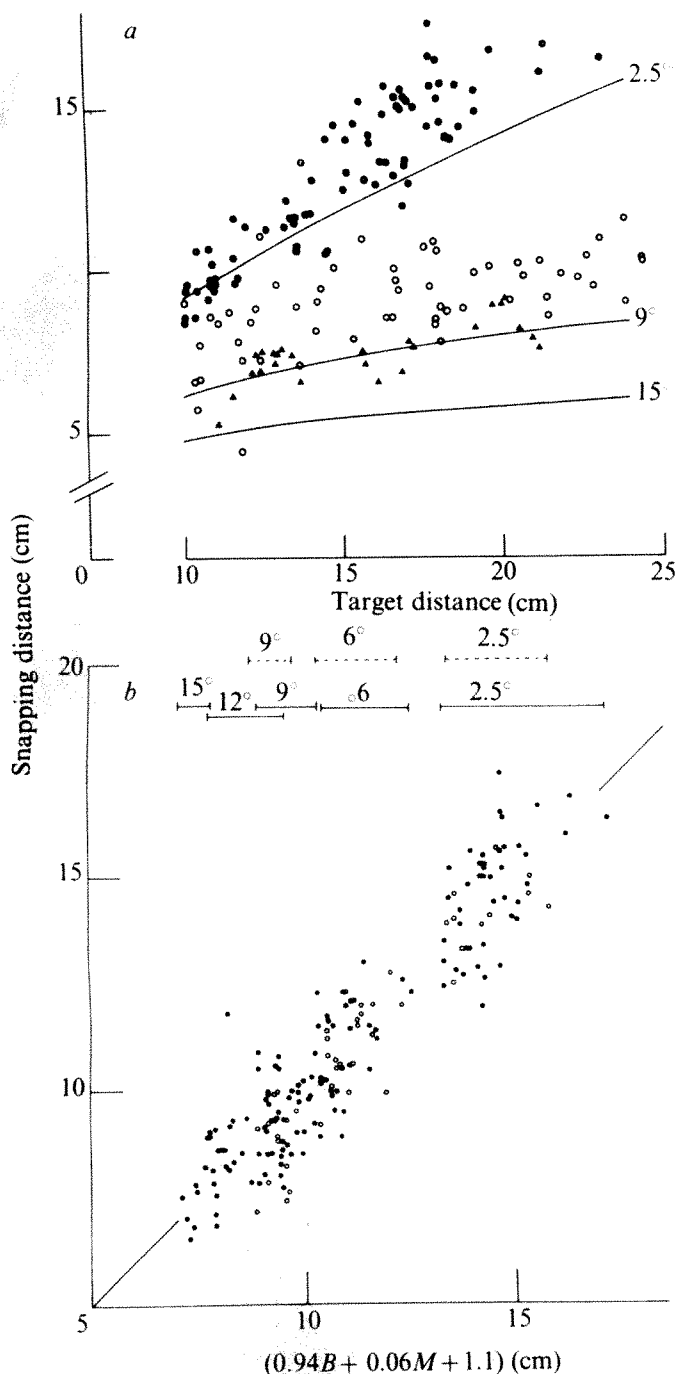


Fig. 3 *a*, Plot of snapping distance against target distance for toads wearing base-out prisms over both eyes. The undershooting caused by the prisms increases with their strength. ●, Minimum angular deviation caused by each prism is 2.5°. ○, Minimum angular deviation is 9°. ▲, Minimum angular deviation is 15°. The solid lines show the predicted snapping distance on the assumption that toads employ stereopsis alone. In these predictions the distance (about 1 cm) between the prism and the eye is taken into account. Data from four toads snapping at a yellow cylinder moved by hand in their frontal visual field. *b*, Relation between *S* and a prediction of *S* derived from the expression $0.94B + 0.06M + 1.1$, with *M* and *B* defined as in Fig. 2*b*. This expression indicates that when targets are viewed binocularly disparity provides the major cue to distance, accommodative information playing a relatively minor role. The predicted value of *S* has been calculated for each data point (from plots like that of Fig. 3*a*) in which the target distance is 15 cm or greater. ●, Snapping responses of toads wearing one of five different prism strengths ($\delta = 2.5^\circ, 6^\circ, 9^\circ, 12^\circ, 15^\circ$). ○, Data points from toads wearing 136 mm lenses and one of three prism strengths (2.5°, 6°, 9°). The lines above the scattergram indicate the range of data points covered by each prism or lens-prism combination. Data from five toads. The goodness of fit of the data to this expression over the whole plot shows that the expression is an acceptable summary of the results.

convergent eye movements, a much greater range of disparities (about 35°) must be measured than is the case in human vision where a relatively narrow but exceedingly sensitive band of disparity detectors can be shifted to any desired position in depth. To confirm that eye movements are not essential for stereopsis in toads the effect of base-out prisms on snapping distance was examined in one toad with its medial and lateral extra-ocular muscles sectioned, and, as expected, base-out prisms continued to cause undershooting.

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Functional test of tight junctions in the mouse blastocyst

THE mouse blastocyst contains a fluid-filled cavity, the blastocoele, which in normal Q-strain embryos first appears at about 83 h post coitum, when cleavage has produced a ball of about 30 cells¹. Two factors could be involved in the formation of the blastocoele: the secretion of fluid, which seems to be brought about by the coalescence and subsequent release of fluid-filled cytoplasmic vacuoles², and the development of tight intercellular junctions. Such junctions are first observed at the eight-cell stage, around the periphery of the embryo³; in the blastocyst they have increased in number and complexity to form the so-called 'zonula occludens', a permeability seal that can exclude lanthanum tracer from the blastocoele and thus presumably plays a role in maintaining the fluid turgor of the blastocyst⁴. By 'immunosurgical' dissection⁵ of Q embryos, we have investigated the stage of normal development at which the permeability seal becomes functional. We report here that impermeability to antiserum was not achieved until the mid-blastocyst stage (45–50 cells), and was not hastened or delayed by experimentally increasing or decreasing total cell number.

The technique of immunosurgery involves incubating embryos in the presence of anti-mouse antiserum; after thorough washing, the embryos are then incubated in complement. Cells coated with antiserum are briskly lysed; interior cells survive if and only if the antiserum has been denied access to them. As well as embryos grown *in vivo*, some aggregated and control cultured embryos were studied, to determine whether the total number of cells in the embryo affects the stage at which antiserum is first excluded. Table 1 shows that antiserum treatment lysed all cells in morulae and early blastocysts, up to 96 h after ovulation. At 97 h, 9% of *in vivo* blastocysts yielded some surviving cells; at 106 h, by which time the embryos were late blastocysts (containing over 100 cells on average), non-lysed cells were recovered from all.

Geometrical considerations suggest that a 17-cell embryo

Table 1 Isolation of inner cell masses from blastocysts of different ages by antiserum treatment

Source	Age (h post coitum)	Estimated mean cell number of embryos†	No. of embryos subjected to immunosurgery	% Embryos with cells surviving	No. of surviving cells Mean†	Range
<i>In vivo</i>	90	45.1*	25	0.0	—	—
	93	52.2*	10	0.0	—	—
	95	57.7*	13	0.0	—	—
	96	60.7*	23	0.0	—	—
	97	67.2(6)	58	8.6	15.0 (2)	15, 15
	106	109.3(7)	33	100.0	35.4 (10)	22–62
<i>In vitro</i> (single)	97	44.6*	27	0.0	—	—
	105	63.4*	71	14.1	21.8 (9)	18–25
	106	66.4*	37	27.0	24.0 (3)	21–27
	110	83.5(4)	38	50.0	20.1 (8)	15–26
	114	94.6*	18	72.2	22.0 (8)	15–28
<i>In vitro</i> (double)	97	—	17	0.0	—	—
	105	—	10	20.0	39.5 (2)	37, 42
	106	—	31	22.6	41.0 (7)	27–57
	112	—	8	75.0	42.6 (3)	38–47
	114	—	23	91.3	37.5 (6)	29–60
<i>In vitro</i> (triple)	114	—	13	100.0	58.3 (7)	48–78

*Estimates of cell number taken from Fig. 2, ref. 1.

†Numbers in parentheses represent the number of observations on which the mean is based.

Spontaneously ovulating females of the randomly bred Q- strain were used, kept in controlled lighting conditions with 16 h light, and 8 h dark centred on either 2400 or 1400. Ovulation and mating were assumed to have occurred at the midpoint of the dark period preceding the finding of the copulation plug. Embryos were either flushed from the uterus on day 4 or 5 of pregnancy (*in vivo*), and the zona pellucida removed with pronase, or recovered from the oviduct on day 3 of pregnancy and grown *in vitro* to a comparable stage of development. Cultures were carried out at 36 °C in a gas-phase of 5% CO₂ in air, under paraffin oil in 20 µl drops of medium 16 (ref. 14) after removal of the zona pellucida. Some of the zona-free eight-cell stages were aggregated into pairs or triplets before culture¹⁵. The method of antiserum treatment was similar to that used by Solter and Knowles⁵. Rabbit antiserum raised to mouse spleen cells was used at a dilution of 1/30 in phosphate-buffered medium¹⁴. Zona-free embryos were incubated in antiserum for 30 min at 36 °C, washed in phosphate-buffered medium at least four times, then incubated in 10% guinea pig complement (Wellcome, lyophilised) in isotonic saline for 30 min at 36 °C. Gentle pipetting dissipated the lysed and dead cells, so that any surviving interior cells could be clearly seen. These were counted after air-drying¹⁶ and staining with lactic-acetic orcein. Cell counts were carried out by the same method on some embryos before antiserum treatment.

should have at least one interior cell⁶. Direct observations have shown that cells may in fact become completely surrounded as early as the eight-cell stage⁷. If peripheral tight junctions formed a fully effective seal when they first appeared, at the eight-cell stage, immunosurgery should allow the isolation of even a single interior cell. It turns out that not only do no morulae survive antiserum treatment, but that the smallest number of cells that can be isolated in this way is about 15 (Table 1). Fifteen interior cells correspond to a blastocyst comprising *in toto* 45–50 cells^{8–10}. By this stage, the blastocyst *in vitro* is known to undergo a series of relatively rapid contractions of its cavity, followed by slower expansions¹¹. The blastocoele fluid seems to be restored and maintained by the action of the trophectoderm^{12–13}, desmosomes between trophectoderm cells are seen for the first time⁴, and the apparent turgor of the expanded blastocyst confirms that a permeability seal is operative. On the other hand in normal development the blastocoele first appears at about the 30-cell stage, before the tight junctions are fully capable of excluding antiserum. Unless the junctions form a one-way seal, impermeable to the passage of fluid from the inside outwards but not yet from the outside inwards, the existence of a fluid-filled cavity at this stage must depend on factors other than a permeability barrier. For example, the cavity could be maintained by a continuous release of fluid from the cytoplasm, or merely by a change in shape of the peripheral cells.

The results for aggregated embryos and their cultured controls are also shown in Table 1. Development *in vitro* was delayed by 7–10 h. Surviving cells were absent at 97 h, and were first observed 105 h after ovulation, both in the single and in the aggregated double blastocysts. By 114 h, non-lysed cells were recovered from the great majority of cultured embryos. The rate at which the proportion of embryos with some cells surviving increased did not differ

significantly between the single and double group, although the number of cells that survived was greater by almost twofold in the doubles than in the singles, and by almost threefold in the triples. The smallest number of cells recovered after antiserum treatment was 15 for single embryos *in vitro* (the same as *in vivo*), 27 for double, and 48 for triple embryos.

Thus, the time at which peripheral tight junctions are capable of excluding antiserum from the interior of the embryo does not depend on the size of the total cell mass. The time of first appearance of the blastocoele was also found to be independent of cell number, and was associated neither with the number of cell divisions nor with the chronological time elapsed since fertilisation¹. For blastocoele formation, the developmental clock could involve the number of DNA or chromosome replications, or possibly changes in nucleo-cytoplasmic ratio. Once blastocoele formation has begun, it may be that fluid pressure from the interior of the embryo is instrumental in the transformation of the semi-permeable system of tight junctions into a true zonula occludens.

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Splenic T-killer cells can be generated by allogeneic thymic cells in conjunction with assisting factor

CELL-MEDIATED lympholysis (CML) is known to be brought about by thymus-derived (T) killer cells that have acquired reactivity towards antigens borne on target cells. To generate such T killer cells, cooperation between two subsets of T cells within the effector population is required—namely, T helper and T pre-killer cells¹⁻³. Helper T cells are thought to recognise Ia antigens^{4,5} and/or unique H-2 antigens⁶, while T pre-killer cells are known to recognise H-2 antigens^{4,5}. Ia antigens are found on B cells as well as on a certain sub-population of T cells⁷, whereas H-2 antigens have been described on virtually all B and T cells⁷. Plate has shown that a soluble factor from alloantigen-activated T helper cells assisted T pre-killer cells to differentiate into killer cells⁸. In this instance, soluble helper factor was generated in separate cultures containing mixtures of allogeneic cells, which was then assayed in a CML system from which T helper cells had been serologically removed. Since most thymic cells are known to lack Ia antigens⁷, they are conceded to be poor stimulators in both CML and mixed lymphocyte reactions⁹⁻¹¹. Nevertheless, they possess H-2 antigens and we reasoned that they might still serve to stimulate the generation of killer cells provided an assisting factor was incorporated from an exogenous source. In this way, T killer cells might be generated without at all disturbing the effector cell population by serological deletions. The data presented here support the idea that thymocytes alone are incapable of activating T helper cells, but that in the presence of exogenously produced assisting factor they do trigger development of T killer cells. The data also indicate a simple way by which assisting factors may be studied and by which requirements for CML may be delineated.

To illustrate the simplicity by which killer cells may be generated using this method, C57BL/6 spleen cells (10^7) were co-cultured, for sensitisation purposes, with allogeneic stimulator cells (10^7 , CBA/H X-irradiated with 2,000 rad) derived from spleen and thymus. Supernatant fluids were added to certain of the cultures containing thymic cells as stimulators, to assist the generation of effector cells. These fluids were added to cultures at the outset of the sensitisation phase in concentrations that ranged from 40 to 50% of the total volume, as noted in the tables. These assisting supernatant fluids were produced by co-culturing 10^7 C57BL/6 spleen cells for 48 h in a 5% CO₂ environment with a similar number of CBA/H spleen cells that had been X-irradiated with 2,000 rad. Such cultures were centrifuged at 170g for 10 min and filtered (0.45 µm Millipore filter) to eliminate viable cells. A control supernatant fluid was produced by culturing 10^7 C57BL/6 spleen cells alone. To test for killer activity, the ⁵¹Cr release assay was performed in microtitre plates according to the method of Simpson³.

CBA/H splenic cells served quite well to stimulate C57 splenic cells to become killers, whereas CBA/H thymic cells did not (Table 1). However, if supernatant fluid from allogeneic mixtures of splenic cells was included in the medium during the sensitisation phase, thymocytes were capable of sensitising the C57 splenic cells to become killers. Killer cells produced were specific only for the sensitising cells (H-2^b) (see Table 1). Supernatant fluids taken from C57 splenic

Table 1 Allogeneic thymic cells serve as stimulators of killer cells provided they are assisted by humoral factor(s)

Sensitisation of C57BL/6 effector spleen cells by CBA stimulator cells* from:	Addition of supernatant fluids† derived from splenic cells	Specific lysis‡ (% ± s.d.)	
		Effector: Target 2:1	Effector: Target 4:1
Spleen	—	41.8 ± 9.8	52.4 ± 10.1
Thymus	—	9.6 ± 7.5	14.0 ± 5.6
Thymus	C57BL/6 + CBA/H*	40.9 ± 15.0	54.3 ± 16.7
Thymus	C57BL/6	8.6§	11.8§

|| After 5 d in culture, viable cells were counted by the trypan blue exclusion method and used as effector cells. Target cells were CBA/H splenic cells that had undergone blastogenesis for 2 or 3 d in the presence of concanavalin A (Miles) and that had been labelled with ⁵¹Cr (3×10^7 blast cells incubated with 100 µCi of sodium chromate for 90 min.). ⁵¹Cr-labelled target cells (10^5) were mixed with 2×10^5 or 4×10^5 effector cells, centrifuged for 5 min at 45 g and incubated for 3 h at 37 °C. Thereafter, the plates were centrifuged for 10 min at 170 µg and 100 µl of supernatant fluid was removed and assayed for released label with a gamma counter. To control for specificity of lysis, C57 effector spleen cells were co-cultured separately with splenic and thymic cells of (C57BL/6 × CBA)F₁ mice. Some of the latter were in the presence of KAF. After 5 d, effector cells sensitised to splenic cells achieved lysis equal to 52.1% and 0.7% against CBA (H-2^a) and C57BL/6 (H-2^b) target cells, respectively. Those effectors sensitised to thymic cells alone gave 10.8 and -2.0%; those sensitised to thymic cells in the presence of KAF achieved lysis of 59.1 and 6.1% respectively. In addition, those effector cells sensitised with CBA spleen failed to significantly lyse DBA/2 targets (H-2^a) (8.9%) as did effector cells sensitised with CBA thymus cells in the presence of KAF (8.0%).

*Exposed to 2,000 rad X-ray irradiation.

†Added at 40-50% of total volume of the cultures.

‡% specific lysis, [(sensitised cells - non sensitised cells/maximum release - spontaneous release)] × 100.

§For these five experiments, the average maximum release was 5,079 c.p.m. and the average spontaneous release was 1,134 c.p.m.

§No s.d. provided as these values are averages of two experiments.

cells cultured alone were not able to help in generating killer cells (Table 1). The inability of C57 spleen cells cultured alone to produce killer assisting factor (KAF) could not be attributable to a reduction in cell viability at the end of the culturing interval (data not shown). An additional control not shown in Table 1 consisted of using no stimulator cells but only assisting supernatant fluid in the sensitising phase. This condition produced no killer cells indicating that both stimulator cells and KAF are essential to the process and that cellular fragments or antigens present in the supernatant fluids were not responsible for generating killer cells.

Table 2 Killer assisting factor (KAF) acts on T cells

Characterisation of Effector cells (C57BL/6)	Stimulator cells* (CBA/H)	Assisting supernatant fluid† added	Specific lysis (%) Effector: Target‡ 4:1
Whole spleen	Whole spleen	0	61.4
	Thymus	0	10.6
	Thymus	+	71.8
Splenic T cells (Nylon wool non-adherent)	Whole spleen	0	67.0
	Thymus	0	4.8
Splenic B cells (Anti-Thy-1.2 + C')	Whole spleen	+	57.6
	Thymus	0	1.0
Whole spleen (Normal mouse serum + C')	Whole spleen	0	n.d.
	Thymus	+	1.8
	Whole spleen	0	67.4
	Thymus	0	22.2
	Thymus	+	76.9

*Exposed to 2,000 rad X-ray irradiation

†From C57 spleen + CBA/H spleen (2,000 rad) added at 40% of the volume of the culture.

‡The maximum and spontaneous releases for this experiment were 3,893 c.p.m. and 544 c.p.m. respectively.

n.d., not determined.

Table 3 T Cells are a source of KAF

Production of supernatants	Sensitisation phase		Effector phase
Subpopulation of C57 spleen cells used to produce KAF§	C57 spleen cells sensitised by CBA cells* from	KAF supernate added†	Specific lysis (%) Effector: Target‡ 4:1
—	Spleen	—	64.8
—	Thymus	—	12.6
Whole spleen	Thymus	+	57.8
Nylon wool non-adherent (T)	Thymus	+	60.3
Anti-Ig + C' (T)	Thymus	+	45.4
Normal goat serum + C' (whole spleen)	Thymus	+	57.5
Nylon wool adherent (B)	Thymus	+	11.4
Anti-Thy-1.2 + C' (B)	Thymus	+	17.8
Normal mouse serum + C' (whole spleen)	Thymus	+	50.7

*Exposed to 2,000 rad X-irradiation.

†50% of total culture volume.

‡Maximum and spontaneous releases were 5,132 c.p.m. and 927 c.p.m. respectively.

§C57 splenic cells were separated into two fractions by passage through nylon wool columns. Other aliquots of C57 spleen cells (40×10^6) were treated with θ -specific anti-serum (Thy-1.2 as in text) plus complement (agarose absorbed guinea pig serum) or with Ig-specific anti-serum (Cappel Labs.) plus complement for 1 h at 37 °C. Some C57 spleen cells were incubated with normal mouse serum plus complement and normal goat serum plus complement as controls. Following these treatments, the remaining viable cells were mixed with X-rayed CBA/H spleen cells to make assisting supernatant fluids, as described in the text.

To determine that T cells were, indeed, the killer cells in this system and to learn whether KAF was acting on killer cell precursors, fractionated splenic T cells and B cells were used separately as the intended effector cells during the sensitisation phase. Fractionated splenic T and B cell populations were obtained using nylon wool columns according to the method of Julius *et al.*¹². Since the nylon wool adherent cell population is known to consist of a mixture of B and some few T cells, this fraction was further treated with anti-Thy 1.2 serum (AKR-anti-C3H at 1:10 final dilution) plus agarose-absorbed guinea pig serum. The results shown in Table 2, which represent one of two parallel experiments, substantiate the known fact that splenic T cells, when confronted with allogeneic spleen cells as stimulators, will kill allogeneic target cells to approximately the same degree as do whole spleen cells acting as effectors (67.0% compared with 61.4%). Furthermore, just as with whole spleen cell populations, splenic T cells did not become effector cells when co-cultured in the presence of allogeneic thymic cells alone (10.6% and 4.8%). Splenic T cells, however, just as whole spleen cells, developed killer T cells when provided the double stimuli of allogeneic thymic cells and KAF (57.6% and 71.8%). Splenic B cells were unable to generate effector cells either in the presence of allogeneic whole spleen cell stimulators (1%) or in the presence of thymic cells and KAF (1.8%). As control, whole spleen cells intended as effectors were exposed to normal mouse serum plus complement prior to sensitisation. That these cells showed a slight increase in their ability to lyse target cells may be attributable to experimental error although other possibilities exist.

Experiments were also conducted to identify the cell source of KAF. This was accomplished by nylon wool separation of whole spleen cell populations and by serologically eliminating sub-populations from spleen cells and using the remainder in conjunction with irradiated allogeneic spleen cells for supernatant fluid production.

When splenic cells were depleted of B cells either with nylon or by anti-Ig serum and complement, the remaining cells produced KAF (Table 3). On the other hand, deleting T cells from splenic populations with nylon wool or serologically, eliminated KAF production. Indeed, these data (lines 7 and 8, Table 3) also show that KAF does not originate from the X-irradiated stimulator cells. Thus, two separate lines of evidence identify the source of KAF as T cells. We do not, however, exclude the possibility that the factor might be produced by other cells under other conditions.

Thus, a new simplified method has been described by

which thymic cells in conjunction with exogenously produced assisting factors will generate killer T cells for CML. KAF has been shown to be produced by T cells that are stimulated by allogeneic spleen cells. KAF was found to act upon T cells during the sensitising phase. Thymic cells alone serving as stimulators in the sensitisation phase, did not generate T killer cells. Exogenous KAF is therefore required as an additional signal when thymic cells are used as stimulators of pre-killer T cells. Whether KAF can be produced by stimulating T helper cells with cells that are not allogeneic, or with mitogens (concanavalin A) is not known. The exact requirements for the generation of KAF in this system as well as its antigenic and strain specificities are currently being investigated.

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Cytotoxic T cells kill influenza virus infected cells but do not distinguish between serologically distinct type A viruses

THE molecular basis of recognition and target cell killing by cytotoxic T cells is still unknown. Doherty and Zinkernagel showed that mouse cells infected with lymphocytic choriomeningitis virus can be lysed by immune cytotoxic T cells but only if both cell types share at least part of the major

histocompatibility (H-2) region (see ref. 1). H-2 compatibility is also required for cell killing in other viral systems²⁻⁴, and for cells carrying non-viral antigens⁵⁻⁷. Our aim is to analyse the virus specificity of cytotoxic T cells, and the nature of their antigen recognition units. This requires a virus system with a small number of well characterised protein components, amenable to biochemical and genetic manipulation, as well as cytotoxic cells that will lyse target cells in a short term assay to minimise nonspecific cytotoxicity. Influenza virus offers many advantages for this type of study. The virion contains only two well characterised surfaced antigens, haemagglutinin (H) and neuraminidase (N), that are also expressed on the surface of infected cells⁸⁻¹¹, and can be purified in quantity. Virus strains are available with serologically distinct surface proteins, and with different internal proteins^{12,13}. Here we describe the generation of potent cytotoxic cells that are specific for histocompatible influenza virus-infected cells, but exhibit extensive cross-reactivity between the different type A influenza virus strains.

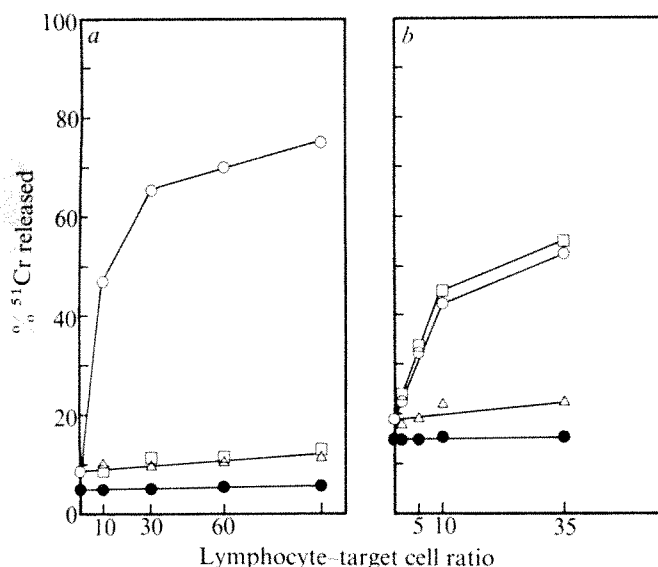


Fig. 1 Cytotoxic T cell-killing of H-2 compatible target cells infected with type A/Jap/Bel influenza virus. ⁵¹Cr release assay was carried out in microtitre II plates⁶, using 2×10^4 lymphoblasts or 10^4 P815 cells and varying numbers of immune cells per well as described by Simpson *et al.*²². Incubation was for 3 h. % ⁵¹Cr release = (counts released by target cells/counts released by 2.5% Triton X-100) $\times$ 100. Induction of cytotoxic cells: spleen cells after removal of red cells by 0.184 M NH_4Cl , were cultured with 2×10^6 stimulator cells for 5 days at 37 °C in Sterilin vials in RPMI 1640 medium supplemented with 10% foetal calf serum (FCS), 5×10^{-5} M mercaptoethanol (ME), and antibiotics (4×10^6 spleen cells in 4 ml per vial). Stimulator cells were syngeneic macrophages infected with type A/Jap/Bel (10^7 peptone-induced peritoneal cells were incubated with 100 HA units type A/Jap/Bel in siliconised tubes for 90 min at 37 °C and washed). Infection of target cells: 10^7 P815 mastocytoma cells (H-2^d, DBA/2) in 1 ml RPMI 1640 were incubated with 100 μCi ⁵¹Cr and 100 HA units type A/Jap/Bel for 90 min at 37 °C, washed three times, and incubated at 10^6 cells per ml in RPMI 1640 + 10% FCS for another 4 h. Lymphoblasts were obtained by stimulating 10^7 spleen cells in 10 ml RPMI 1640 medium supplemented with 10% FCS, 5×10^{-5} M ME and antibiotics for 2 d with 10 μg *E. coli* lipopolysaccharide in 25 ml Falcon flasks. Large blast cells were purified on a BSA gradient²¹ and labelled and infected as above. *a*, BALB/c spleen cells, cultured with macrophages for 5 d and assayed on ⁵¹Cr-P815 target cells. (Δ), Normal (unprimed) spleen cells, stimulated with infected macrophages, assayed on infected target cells; spleen cells from intranasally primed mice cultured with infected macrophages ($\circ$), or cultured with uninfected macrophages ($\square$), assayed on infected target cells; ($\bullet$) as ($\circ$), but assayed on uninfected target cells. *b*, CBA/H spleen cells (from intranasally primed mice) were cultured with infected macrophages and assayed on ⁵¹Cr-CBA/H lymphoblasts. ($\circ$) infected target cells; ($\bullet$) uninfected target cells; (Δ) cytotoxic cells treated with AKR anti-Thy 1.2²³ + C', assayed on infected target cells; ($\square$) cytotoxic cells treated with normal AKR serum and C', assayed on infected target cells.

An *in vitro* system for the secondary induction of spleen cells from primed mice¹⁴⁻¹⁸ was used to generate cytotoxic T cells which lysed influenza virus-infected cells in a 3-h assay. Virus was grown in embryonated chicken eggs and allantoic fluid was used as inoculum. Mice (2-3 months old) were primed by infecting them intranasally on two consecutive days with 4 HA units (0.05 ml) of influenza virus strain A/Jap/Bel (WHO nomenclature¹⁹); 5-12 weeks later spleen cells were put into culture with infected syngeneic macrophages for the *in vitro* generation of cytotoxic cells. After 5 d of culture, the cells were assayed for their ability to lyse ⁵¹Cr-labelled target cells infected with different strains of influenza virus. Either histocompatible P815 mastocytoma cells (H-2^d, of DBA/2 origin) or syngeneic lymphoblasts activated by *Escherichia coli* lipopolysaccharide (LPS) served as target cells. The latter were purified from bovine serum albumin gradients²¹, since virus replication occurred in blast cells only. We tested a number of mouse strains, but report only results with cytotoxic cells from CBA/H (H-2^k) or BALB/c (H-2^d) mice.

Primed BALB/c spleen cells cultured for 5 d with infected syngeneic macrophages yielded cytotoxic cells which lysed P815 cells infected with type A/Jap/Bel, but not uninfected P815 cells (Fig. 1a). Similarly, primed CBA/H spleen cells killed infected lymphoblasts, but not uninfected cells (Fig. 1b). Over a number of experiments the spontaneous release of ⁵¹Cr from infected P815 tumour cells was lower (5-15%) than that from infected lymphoblasts (15-30%); and the latter cells showed lower specific maximum ⁵¹Cr release. Lymphoblasts, however, have the considerable advantage of permitting studies of humans and outbred animals. The *in vitro* generation of the virus-specific cytotoxic cells requires the priming of spleen donors and the presence of virus-infected macrophages (Fig. 1a). The virus-specific cytotoxic cells are thymus-derived cells, since treatment with AKR antiserum to Thy 1.2 and complement²³ abolished the cytotoxic activity of CBA/H cells, whereas normal AKR serum and complement had a negligible

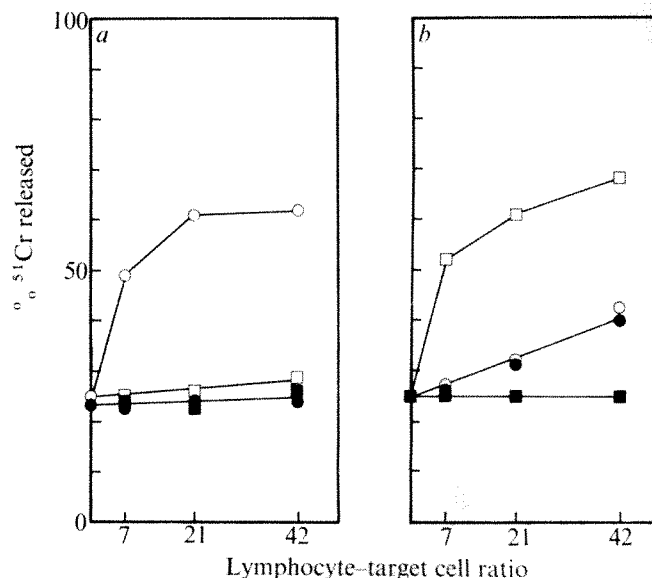


Fig. 2 H-2 restriction of cytotoxic killing. Experimental details, as for Fig. 1. BALB/c and CBA/H cytotoxic cells (CT) were generated *in vitro* by incubating spleen cells from mice that were primed with type A/Jap/Bel with syngeneic macrophages infected with type A/Jap/Bel. Syngeneic or allogeneic ⁵¹Cr-labelled lymphoblasts (LBL) were used as target cells. *a*, BALB/c CT cells assayed on infected BALB/c LBL ($\circ$), or uninfected BALB/c LBL ($\bullet$); CBA/H cytotoxic cells assayed on infected BALB/c LBL ($\square$), or uninfected BALB/c LBL ($\blacksquare$). *b*, BALB/c CT cells assayed on infected CBA/H LBL ($\circ$), or uninfected CBA/H LBL ($\bullet$); CBA/H CT cells assayed on CBA/H infected LBL ($\square$), or CBA/H uninfected LBL ($\blacksquare$).

effect (Fig. 1b). Similar results were obtained when BALB/c cells were treated with anti-Thy 1.2 (not shown).

The killing of influenza virus cells showed H-2 restriction similar to that observed with other viral systems¹⁻⁴. BALB/c (H-2^d) cytotoxic cells killed infected syngeneic BALB/c lymphoblasts but not infected allogeneic CBA/H (H-2^k) lymphoblasts (Fig. 2). Conversely, CBA/H cytotoxic cells killed infected CBA/H but not BALB/c lymphoblasts. A low level of cytotoxic activity was observed by the BALB/c lymphoblasts against allogeneic CBA/H lymphoblasts, but this cytotoxicity was directed equally to uninfected as well as infected cells.

The specificity of recognition by the virus-specific cytotoxic T cells was assayed using H-2 compatible target cells that were infected either with heterologous strains of type A influenza virus A/Jap (H₂N₂), A/Bel (H₀N₁), A/X31 (H₃N₂) (ref. 20) or with a B strain of influenza virus (B/Hong Kong). The type A viruses share internal antigens with type A/Jap/Bel (H₂N₁) but contain either serologically distinct haemagglutinins (H₀ or H₃) and/or neuraminidases (N₂). Note that type A/X31 does not share any surface antigens with type A/Jap/Bel, and that types A/Jap and A/Bel share the haemagglutinin or neuraminidase respectively. The type B/Hong Kong differs from the type A strains in both internal and surface antigens. BALB/c cytotoxic cells sensitised against type A/Jap/Bel lysed P815 cells infected with type A/Jap/Bel, A/Jap, A/Bel and A/X31 with equal efficiency, but did not lyse cells infected with type B/Hong Kong (Fig. 3). Conversely, incubation of spleen cells from B/Hong Kong primed mice with B/Hong Kong infected macrophages generated cells cytotoxic for B/Hong Kong infected but not A/Jap/Bel infected P815 cells (data not shown). We obtained the same specificity results when infected CBA/H lymphoblast cells served as target cells, and when cells were infected with another type B virus (B/Lee).

Our results demonstrate that T cell-mediated cytotoxicity of cells infected with influenza virus shows H-2 restriction as previously described for other virus systems¹⁻⁴. Of special

interest is the finding that at least a major proportion of the cytotoxic T cells are unable to discriminate between different type A influenza virus strains, whereas humoral antibodies can readily distinguish the haemagglutinin and neuraminidase proteins of these type A viruses. The T cell killing is virus-specific, since cells infected with type B influenza virus are not recognised by the cytotoxic cells generated with type A virus. Our results in the secondary response differ from those of Cambridge *et al.*²⁴, who report that a primary cytotoxic response is specific for the viral haemagglutinin in an 18-h assay. During the preparation of this manuscript we learned the Doherty and collaborators have observed cross reaction between type A viruses similar to our findings²⁵.

There are several possible explanations for the apparent difference in T and B cell recognition. T cells may recognise one of the shared internal viral proteins, provided it is expressed at the surface of infected cells. Alternatively, if the surface proteins (haemagglutinin and/or neuraminidase) are involved in T cell-recognition, the T cells would need to recognise a shared region of these surface proteins which for some reason is not immunodominant for B cells. Viral proteins expressed at the surface of infected cells may have a different conformation from those expressed at the surface of virions, resulting in the exposure of a shared region. It is also possible that T cell-mediated cytotoxicity is a more sensitive method of detecting cross reactivity than humoral antibody assays. Further studies with purified viral proteins will enable us to differentiate between these possibilities. Whatever the molecular basis of our observations, the cross reactivity for type A influenza virus strains at the T cell level is of obvious importance in immunisation against and the immunopathology of viral infections.

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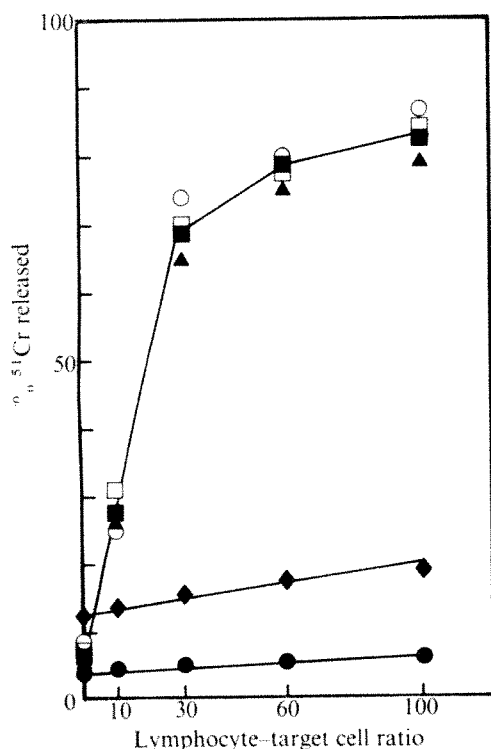


Fig. 3 Virus specificity and cytotoxic killing. Experimental details as for Fig. 1. BALB/c cytotoxic cells were generated against influenza virus type A/Jap/Bel. Target cells were ⁵¹Cr-P815 cells infected with the following strains of influenza virus: ○, A/Jap/Bel (H₂N₁); ▲, A/X31 (H₃N₂); □, A/Jap (H₂N₂); ■, A/Bel (H₀N₁); ◆, B/Hong Kong; ●, uninfected.

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***In vitro* induction of immunological memory against testicular autoantigens**

By culturing normal rodent lymphocytes together with foreign stimulator cells, lymphocytes are generated, which can destroy target cells bearing transplantation antigens identical with the ones on the stimulator cells¹⁻³. When these stimulated lymphocytes are cultured further in the absence of the primary cellular antigen, they will revert, morphologically, to small lymphocytes⁴⁻⁶. These primed 'secondary' (2°) lymphocytes⁷ are functionally oligoclonal memory cells which can be reactivated only by stimulator cells, which resemble, antigenically, the ones that caused the primary stimulation. We have studied the occurrence of a parallel phenomenon with self, rather than foreign, tissue and have established that normal rat lymph node cells can be auto-sensitised *in vitro* against dissociated testis cells from the same donor animal⁸. I report here that the self responsive activated lymphocytes can also be made to revert back to quiescent small secondary lymphocytes

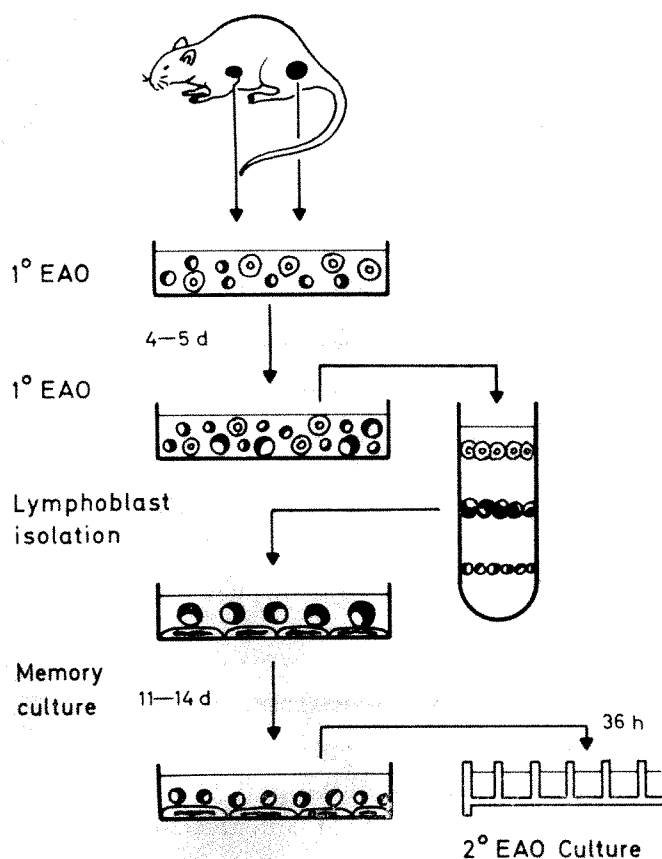


Fig. 1 *In vitro* generation of auto-sensitized EAO secondary cells. For primary auto-sensitisation 30×10^6 normal rat lymph node lymphocytes and 3×10^6 trypsin dissociated testis cells were incubated in 6 ml medium (Eagle's medium + 15% heat inactivated horse serum). After 4-5 d, the stimulated cultures were fractionated on a discontinuous Ficoll density gradient. The gradient consisted of five layers with decreasing density varying from 1.10 to 1.06 g ml⁻¹, and a top layer of Ficoll-free Eagle's medium. Centrifugation was at 10,000 r.p.m. (Beckman head SW27) for 60 min at 4°C. The purified blast fractions, containing more than 95% lymphoblasts, were seeded together with allogeneic embryonic fibroblasts (2×10^6 lymphoblasts, 1×10^6 fibroblasts) in 10 ml culture medium into 100 mm Petri dishes. After 11-14 d, the reverted small 2° lymphocytes were collected and confronted with fresh stimulator cells in microtitre plates (2×10^5 2° lymphocytes plus 1×10^6 irradiated lymph node stimulator cells (2,000 rad), or 1×10^5 dissociated testis cells, respectively). For determination of ³H-thymidine uptake, ³H-thymidine was added 24 h after starting the 2° cultures, and cells were collected in a multiple harvester after overnight incubation.

and that immunological memory can be induced *in vitro* against autoantigens.

The *in vitro* system used is a tissue culture correlate of experimental autoimmune orchitis⁸. In mixed cultures of fresh lymphocytes and autologous, trypsin dissociated testis cells, lymphoblasts appear within 4-5 d, which can induce progressive orchitis when reinjected into syngeneic recipients⁸, and *in vitro* specifically destroy syngeneic target cells (H.W., unpublished).

Following the protocol outlined in Fig. 1, I isolated the autoreactive lymphoblasts and allowed them to revert back to small secondary lymphocytes. The cellular events taking place in secondary EAO cultures, containing secondary lymphocytes plus relevant stimulator cells, are remarkable. In such cultures, large aggregates of lymphocytes and testis cells are formed within 12 h. Within 24 h of culture, most of the small lymphocytes have been retransformed into lymphoblasts. Cultures containing secondary EAO lymphocytes together with allogeneic testis cells, however, do not develop a demonstrable lymphocyte response.

These morphological events are exactly paralleled by the ³H-thymidine uptake observed in the cultures. The data in Table 1 represent one of six consecutive experiments. They demonstrate that L.BDV secondary lymphocytes which were primed against autologous testis cells respond strongly when exposed to syngeneic L.BDV testis cells.

Table 1 MHC specific restimulation of 2° lymphocytes which were primarily auto-sensitized against autologous testis cells

Stimulators in 2° cultures	Responder cells	
	Cell type	2° EAO-L.BDV lymphocytes
Testis	L.BDV	30'956 ± 2'380
	Lewis	4'305 ± 945
	L.BDV	114'507 ± 9'306
Lymphocytes	Lewis	13'485 ± 2'535
	Fresh L.BDV lymphocytes	1'510 ± 257
		2'247 ± 141
		1'834 ± 298
		6'305 ± 325

L.BDV lymphocytes were *in vitro* auto-sensitized against autologous trypsin dissociated testis cells. 2° lymphocytes which had been cultured for 11 d in the absence of external autoantigen were confronted in microtitre cells with syngeneic L.BDV and MHC-congenic (with different MHC, but identical genetic background) Lewis testis cells, and irradiated lymph node lymphocytes. In parallel cultures 1×10^6 fresh L.BDV lymphocytes were incubated with the four types of stimulator cells.

The same secondary lymphocytes do not react against allogeneic Lewis testis cells. These results demonstrate the immune specificity of the auto-sensitized secondary EAO lymphocytes. Furthermore, since L.BDV and Lewis rat strains are congenic with respect to their major histocompatibility gene complex (MHC), sharing an identical genetic background, and differ only at the MHC, these data, together with results obtained in other congenic combinations (for example, Table 2), suggest that MHC autoantigens have been recognised in the secondary reaction.

Unexpectedly, 2° EAO lymphocytes were not only re-stimulated by syngeneic testis cells, but also, to an even higher rate, by syngeneic lymph node cells (Table 1). Fibroblasts, on the other hand, did not elicit 2° responses. Two explanations may account for this phenomenon. First, lymphocytes may share cross-reacting autoantigens with testis stimulator cells. On the other hand, it is possible that the primary EAO reaction is the sum of two autoimmune components, one directed against testis cells, and the other against some cellular subpopulation among the lymph node cells. Accordingly, in the 2° reaction, the lymphocyte clones reacting with testis stimulators should be distinct from those activated by syngeneic

lymphocytes. Indeed, such 'spontaneous' reactions have been repeatedly described⁹⁻¹².

What is the nature of the autoantigens that elicit the secondary EAO reaction? The results shown in Table 1 suggest that antigens of the MHC are involved in the reaction. These H-1 antigens may be recognised in their native state, as 'unaltered self', by the secondary lymphocytes. On the other hand, these determinants may have been modified artificially by the culture procedures. In the latter case, the EAO reaction would correspond to lymphocyte reactions directed against syngeneic stimulator cells that have been altered either chemically¹³, by virus infection¹⁴ or by tumour transformation¹⁵.

The primary EAO reaction observed was directed against syngeneic testis cells which were dissociated by trypsin digestion. This treatment may either change normal surface antigens present on the surface membranes, or expose normally hidden determinants¹⁶. The finding that 2° cells primed with trypsin-digested testis cells can react optimally with syngeneic stimulator lymphocytes which have not been chemically treated at any time point, argues against this possibility. Moreover, a 2° reaction can also be readily initiated by testis cells that were mechanically and not enzymically dissociated.

Table 2 Lack of effect of heterologous culture sera on the specificity of the 2° reaction.

Stimulator cells	Horse serum	Serum additives Foetal calf serum
L.AS2	10,157 ± 764	10,752 ± 1,164
Lewis	1,210 ± 251	1,892 ± 191

L.AS2 lymphocytes were primarily autosenesitised against autologous testis cells, and reverted to 2° lymphocytes in the presence of horse serum. The washed 2° lymphocytes were tested for their immune specificity in the presence of either horse serum or foetal calf serum. The antigens in the 2° reactions were syngeneic or congenic freshly dissociated testis cells.

Another possible cause of 'self modification' may be the heterologous serum present in the culture medium. Several investigators have taken pains to show that horse serum contains components that will readily bind to mammalian cell surfaces^{17,18}. Such heterologous serum proteins could act as the foreign determinants required for an 'altered self' type of reaction. We tested this possibility by confronting 2° EAO lymphocytes (strain L.AS2), which had been cultured in horse serum medium, with syngeneic L.AS2 and MHC congenic Lewis stimulator cells either in medium containing horse serum, or, in parallel, in medium substituted with foetal calf serum. Medium supplemented with autologous serum could not be used, since this serum contains factors which specifically interfere with self recognition¹⁹. As shown in Table 2, the supplementing sera did not affect the specificity of the 2° reactions. Since it is probable that horse and foetal bovine serum proteins do not exhibit marked immunological cross-reactivity, alteration of MHC antigens by heterologous serum proteins does not seem to be a cogent explanation for the *in vitro* EAO. Moreover, it should be noted that, if foreign serum components played a critical role in the EAO reaction, the 2° lymphocytes, which possess normal amounts of MHC antigens themselves, should stimulate each other without the addition of any further target antigens.

Another possible explanation for self alteration is neo-expression of latent virus antigens on the stimulator cells. This possibility cannot be formally excluded at present and, indeed, there are claims that mouse lymphocytes can spontaneously express virus antigens after 24 h of culture²⁰. It should, however, be emphasised that in

secondary cultures the first signs of immune recognition and reaction against freshly isolated stimulator cells were noted within the first few hours of contact. This is a time span hardly sufficient for spontaneous and regular expression of virus antigens.

In conclusion, the data reported here lend further support to the notion that the normal immune system contains T lymphocytes, which are potentially able to react against the body's own tissues²¹. Our autoimmune memory system offers the opportunity to directly investigate the clonally enriched lymphocytes that possess receptors for autoantigen, and may thus be a valuable tool for elucidating the physiological mechanisms responsible for self tolerance at the T cell level. A final implication of our system is more of clinical relevance. Since the autoimmune secondary cells generated *in vitro* have the capacity to discriminate exquisitely between self and non-self, and since this capacity is not lost by storage in liquid nitrogen (unpublished), a similar, though modified, system might be helpful for human tissue typing. A particular advantage of this system is that the results of the reaction can be read after less than 24 h.

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Role of receptor aggregation in complement-dependent inhibition of lymphocytes by high concentrations of concanavalin A

EVIDENCE from haemolytic systems suggests that complement activation by either the classical or alternative pathways requires a close approximation of critical molecules at the cell surface. At least two molecules of IgG antibody in close proximity are required for the first component of complement to be bound¹. Thus when increasing concentrations of IgG are added to erythrocytes in the presence of excess complement, haemolysis is minimal until a critical IgG concentration is reached. In the case of the alternative (properdin) pathway of complement activation, at least two molecules of C3b must be critically orientated together at the cell surface in order for properdin to bind and stabilise C3/C5 convertase². Thus erythrocytes have a simple mechanism of signal discrimination by which they can 'count' the number of molecules reacting with their surfaces. The cells only 'respond' by lysis when a critical number has been reached.

I present data which relate this mechanism of signal discrimination by erythrocytes to the mechanism by which cultured lymphocytes discriminate between low and high concentrations of the mitogenic lectin concanavalin A (con A). This in turn may be related to the mechanism by which lymphocytes discriminate between low and high concentrations of specific antigen to produce either an immune response or immunological tolerance³.

Low concentrations of con A activate cultured rat lymph-node cells (enhanced incorporation of ³H-uridine), whereas high concentrations inhibit the cells by a complement-dependent process^{4,5}. The tetravalent con A molecules are able to aggregate surface receptors for con A on murine and human lymphocytes, but a divalent derivative, succinyl-con A, does not have this property⁶. Furthermore, although both forms of con A, at low concentrations, are able to activate lymphocytes to transform and incorporate ³H-thymidine after 2–3 d of culture, they differ in their effects at high concentrations. Lymphocyte transformation as assessed with ³H-thymidine is inhibited by high concentrations of the tetravalent molecule, but this is not found with the divalent derivative (see ref. 7 for review).

These results suggested that receptor aggregation might be necessary for the complement-dependent inhibition by high concentrations of tetravalent con A. To examine this hypothesis, the effects of various concentrations of succinyl-con A and con A were compared in the presence and absence of complement (bovine calf serum preheated at 56 °C for 30 min). The culture period was only 6 h and this would be expected to reduce the possibility that the cultured cells themselves could secrete complement components in quantities sufficient to influence the results⁸. The succinyl-con A was prepared from con A (Pharmacia) by the double derivatisation procedure described by Gunther *et al.*⁹.

Figure 1 shows that in unheated serum incorporation of ³H-uridine was stimulated at low con A concentrations but inhibited at high con A concentrations. The latter inhibition was not detected in cultures containing serum preheated at 56 °C. This confirms our previous results^{4,5}. In the case of preheated serum, the dose-response curve for succinyl-con A was similar to that for con A except for a small, but consistent, shift of the ascending limb to the right. In the case of unheated serum, the profound inhibition observed at high concentrations of con A was not observed at high concentrations of succinyl-con A. At such concentrations of succinyl-con A the curve plateaued at approximately the same level as the peak response of the curve for con A in unheated serum.

If receptor aggregation were necessary for the complement-dependent inhibition by con A, then both of the dose-response curves for succinyl-con A should have been similar to the curve for con A in heated serum. The depression of the plateau in the case of succinyl-con A in unheated serum suggests that our preparations of succinyl-con A still retain some complement-dependent inhibitory activity; but, even so, the inhibition was much less than that produced by tetravalent con A in unheated serum. Since the response to lectins is quantitatively dependent on the lectin : serum concentration ratio^{5,9}, a possible explanation for the shift of the ascending limbs of the curves for succinyl-con A to the right of those for con A, is that the capacity of serum to bind succinyl-con A is greater than its capacity to bind con A. Cells may also show a reduced binding capacity for succinyl-con A (refs 10, 11).

Although slightly higher concentrations may be required, succinyl-con A has the same capacity at tetravalent con A to activate lymphocytes, but has much less capacity to produce high dose inhibition. Since succinyl-con A produces less receptor aggregation ('patching' and 'capping') than con A, this suggests that such aggregation is not necessary for lymphocyte activation^{12,13}, but is necessary

for lymphocyte inhibition dependent on complement. In apparent conflict with this view is a report that, whereas 'low' concentrations of con A encourage receptor aggregation, 'high' concentrations impede receptor mobility and prevent aggregation¹⁴. Others have found, however, that 'high' concentrations of con A aggregate the receptors both of lymphocytes¹⁵ and of Chinese hamster ovary cells¹⁶. In the latter case cell death occurred. Since the effective concentration of con A depends on the con A : serum ratio⁵, some of these discrepancies may be the result of different serum concentrations employed by different workers. For example, Edelman *et al.*⁶ compared the con A dose-dependence of changes in receptor aggregation using lymphocytes in medium containing albumin (which binds con A weakly), with the con A dose-dependence of changes in cell proliferation using lymphocytes in medium containing serum (which binds con A strongly¹⁷). A concentration of con A which would have been effectively 'low' in the cell proliferation assay would have been effectively 'high' in the aggregation assay. Another apparent discrepancy in the literature is evident from reports that high-dose inhibition by con A is reversible^{18,19}. If complement is involved then such inhibition would be expected to be irreversible. There are a

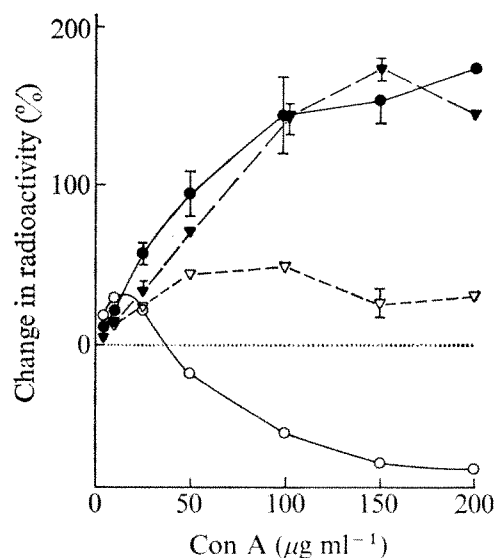


Fig. 1 Comparison of con A dose-response curves (○, ●) with succinyl-con A dose-response curves (▽, ▼) for cultures containing serum which had been preincubated for 30 min at either 38 °C (open symbols) or at 56 °C (closed symbols). Lymph-node cells (2.1×10^6) from female Wistar rats were cultured as described before⁵ in 1 ml of medium containing bovine calf serum (20%), medium 199 (80%) and 1 μ Ci of 5-³H-uridine. After 6 h at 38 °C in a humidified atmosphere of air : CO₂ (95 : 5), cells were harvested for the determination of acid-precipitable radioactivity. Each point is the arithmetical mean of duplicate cultures. In the case of points presented without the standard error of the mean, the limits fell within, or close to, the area covered by the point. Values (c.p.m.) for control cultures without lectin (quadruplicates) were $10,425 \pm 357$ (serum preincubated at 38 °C) and $13,066 \pm 599$ (serum preincubated at 56 °C).

number of technical explanations for the discrepancy some of which have appeared in the recent literature^{20,21}. For example Moller²⁰ has pointed out that in earlier studies¹⁸ he and his co-workers had examined the mitogen-reactivity of cells surviving preincubation for a day with high con A concentrations; the lethal effect on the non-surviving cells had been ignored. In addition, heated calf serum was used; this would have reduced the extent of 'early-onset' inhibition (see below). Recently McClain and Edelman²² carried out similar studies with preheated calf serum. They presented evidence for 'delayed-onset' inhibition (see below) appearing after a day of culture at high con A concentrations. Again, however, attention was focused on the cells

able to survive the inhibition, rather than on the inhibition itself.

The role of complement in lectin-induced lymphocyte inhibition was predicted in 1966 but experiments at that time were negative²³. The eventual demonstration of the role of complement required the use of heterologous (bovine foetal or calf) serum rather than autologous serum^{4,5}. My recent studies indicate that complement inhibitors in autologous serum prevent the onset of the complement-dependent inhibition until after the first day of culture; addition of cobra venom factor to activate the alternative (properdin) pathway can apparently by-pass these complement inhibitors and make complement components available in autologous serum to mediate the inhibition by high con A concentrations in the early hours of culture²⁴. If heterologous serum is preheated the complement-dependent inhibition is not seen in the early hours of culture (Fig. 1). However, an inhibition appears after the first day of culture⁵; we have speculated⁵ that such inhibition requires the secretion of complement components by the cultured cells⁵.

In summary, I have shown that the previously reported failure of high concentrations of succinyl-con A to inhibit lymphocytes as assessed by ³H-thymidine incorporation after 2–3 d of culture⁶ is also demonstrable during the first few hours of culture (³H-uridine incorporation). While the inhibition by tetravalent con A, as assessed with ³H-thymidine, has neither been proven nor disproven to require complement (either present in serum or secreted by cells⁵), the inhibition occurring early in culture has been shown to be complement-dependent^{4,5}. My data thus support the hypothesis that divalent succinyl-con A does not inhibit because it is unable to produce the degree of aggregation of certain molecules at the cell surface which is necessary for complement-dependent inhibition. New techniques, such as resonance energy transfer²⁵, now permit a more detailed examination of the effects of con A and its derivatives on receptor distribution. It would be of interest to correlate the results of such studies with the degree of complement-dependent inhibition at a given con A concentration.

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Use of ionophore A23187 to measure and to control free and bound cytoplasmic Mg in intact red cells

CYTOPLASMIC magnesium, whether free or complexed to nucleotides, has a fundamental physiological role as an essential cofactor for many cell enzymes, particularly those concerned with glycolysis, respiration and membrane transport. Various ingenious procedures have been applied to estimate^{1,2} or simulate^{3,4} intracellular conditions in relation to Mg but, because it is impossible to assess and control Mg²⁺ in the intact cell, most research on Mg requirements for metabolism and transport has used lysed cells⁵ and broken membrane preparations^{6,7}, which have inherent uncertainties about true concentrations and sidedness of effects. Using a divalent cation ionophore⁸ and a newly developed method^{9,10} we have investigated Mg buffering in intact human red blood cells and have found that the fresh, oxygenated, inosine-fed cell (a condition frequently used for ion transport studies^{11–14}) has three main buffer systems which bind nearly 90% of the total Mg present inside the cell in physiological conditions.

Net Mg movements across the intact red cell membrane are unmeasurably slow. Buffering was therefore explored using a selective divalent cation ionophore, A23187, in the absence of other divalent cations. Incorporation of this ionophore into the red cell membrane renders the cell highly permeable to Mg (refs 9, 10) allowing rapid equilibration of Mg ions across the membrane and with the intracellular buffers. By investigating the equilibrium distribution of Mg at different external Mg concentrations (see Fig. 1) a buffering curve can be constructed (Fig. 2). At electrochemical equilibrium the distribution of Mg ions is given by

$$\text{Mg}_i^{2+} = r^2 \text{Mg}_o^{2+} \quad (1)$$

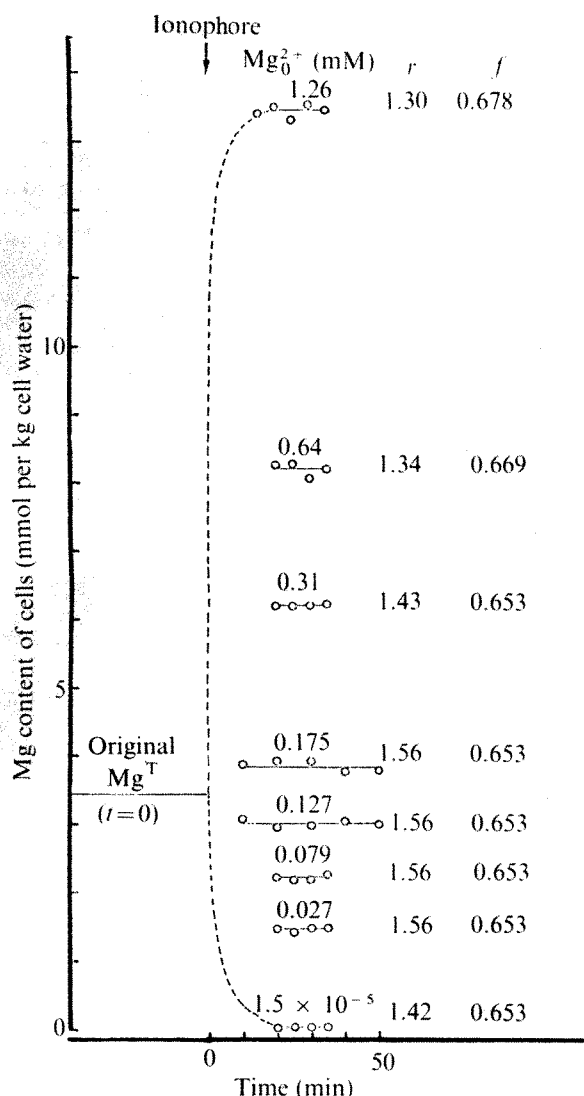
where Mg_i²⁺ and Mg_o²⁺ are the molal concentrations of ionised Mg in cell water and in the medium, respectively, and *r*² is the Donnan distribution ratio for divalent cations at equilibrium. This is related to the membrane potential (*V*_m) by the expression *r*² = exp(–2*FV*_m/*RT*), based on the assumption that the activity coefficients of Mg are identical on both sides of the membrane. The total Mg content of the cells is the sum of the free and bound forms

$$\text{Mg}^T = \text{Mg}_i^{2+} + \text{Mg}^B \quad (2)$$

where Mg^T and Mg^B are the molal concentrations of total and bound Mg in cell water. From equations (1) and (2) we obtain

$$\text{Mg}^B = \text{Mg}^T - r^2 \text{Mg}_o^{2+} \quad (3)$$

The total Mg content of the cells was measured by atomic absorption spectroscopy (Pye–Unicam, SP 90) in the trichloroacetic acid supernatant of cell lysates. In order to estimate Mg^T, Mg^B and *r*², the cell water content and the membrane potential were determined at each Mg concentration, since we had found in preliminary experiments that the relatively large shifts in internal Mg concentration induced in the present experiments had an effect on cell water and on the concentration of non-diffusible anion responsible for the Donnan potential. Cell water was measured as the difference between wet and dry weights of packed-cell samples corrected for extracellular fluid using ⁶⁰Co³⁺–EDTA (ethylenediamine–NNN'N'–tetra-acetic acid) as a marker. The membrane potential was estimated from the ³⁶Cl distribution ratio¹⁵ at equilibrium, *r* = Cl_o[–]/Cl_i[–],



where Cl_o^- and Cl_i^- are the molal concentrations of Cl^- in the external solutions and cell water, respectively (for details see Fig. 1).

The concentrations of internal Mg at equilibrium with different external Mg levels are shown in Fig. 1. The equilibrium distribution of Mg was unaffected by the concentration of ionophore used, indicating that the ionophore neither contributed to nor affected the buffering of Mg by the cell.

Figure 2 shows the results obtained in four different experiments where the equilibrium concentrations of bound Mg (Mg^B) obtained from Fig. 1 and equation (3) are plotted as a function of Mg_i^{2+} . Although the data are not precise enough to allow a fine distinction between individual buffers or between various similar hypothetical models, the simplest model giving a good fit requires the presence of at least three different intracellular buffer systems: (1), about 100 μ mol per kg cell water of very high affinity buffers which are already saturated with Mg when Mg_i^{2+} is about 3×10^{-8} M; (2), about 2 mmol per kg cell water of high affinity buffers, apparent dissociation constant ~ 25 –50 μ mol per kg water; and (3), a large capacity, low affinity buffer system which may bind >1 mol Mg per mol of buffer. About 28 mmol per kg water of a buffer with an apparent dissociation constant ~ 4 mM fits the upper points reasonably well, but the fit of the lower points is improved if this component is attributed a capacity of ~ 20 mmol per kg water and two binding sites with apparent dissociation constants ~ 1 mM.

The original Mg content of the cells used in the present experiments was 3.46 ± 0.05 mmol per kg cell water. From curves such as those in Fig. 2, the Mg_i^{2+} at equilibrium with any value of Mg^T can be obtained. In this way the Mg_i^{2+} in the

Fig. 1 Mg content of cells as a function of time at different external Mg concentrations in the presence of the divalent cation ionophore A23187. Red cells from fresh blood were washed three times with about 10 vol of a solution containing NaCl, 75 mM; KCl 75 mM; Tris-Cl (pH 7.5 at 37 °C) 10 mM; and Tris-EGTA (1,2-di(aminoethoxy)ethane-*NNN'*-tetra-acetic acid) 0.1 mM and three more times with the same solution containing only 0.01 mM or no Tris-EGTA. After washes 0.25 ml of packed cells (2,000g for 5 min) were added to 2 ml of a similar medium also containing inosine (10 mM) and various concentrations of Mg and Tris-EDTA, to yield, together with Mg displaced from the cells, the final concentration of Mg_o^{2+} quoted on the figure. Before addition of ionophore, a 0.1-ml sample was taken to measure the original Mg content of the cells. At $t = 0$, 10 μ l of absolute ethanol containing 1 mg ml⁻¹ of ionophore A23187 were added to the cell suspension under vigorous magnetic stirring. At the times indicated 0.1-ml samples were withdrawn and the cells quickly separated through *n*-butyl-phthalate (BDH; density 1.042–1.045) as described previously^{9,10}, lysed in distilled water, the proteins precipitated with TCA and the Mg concentration measured in the supernatant. Water content and the ³⁶Cl distribution ratio, r , were measured as described in the text and their values are shown. The water fraction (f) is expressed as the weight of evaporated water per unit volume of packed cells. The volume of the extracellular fluid was $2.25 \pm 0.03\%$ of the volume of the cell pellet. The external concentration of Mg_o^{2+} at equilibrium was estimated from the nominal concentrations of Mg and EDTA in the original medium and the redistribution of intracellular Mg. The nominally Mg-free medium contained 2 mM Tris-EDTA and no added Mg; at equilibrium, Mg_o^{2+} was about 1.5×10^{-8} M and Mg_i^{2+} about 3×10^{-8} M. Variations in the external Na and K concentrations, and preincubation of the cells with inosine (variations used as part of other studies on the Mg dependence of active Na transport which will be reported elsewhere) had no effect on the Mg distribution. For clarity, only representative values for each concentration range are shown on the figure. The equilibrium concentration of intracellular Mg was measured for various lengths of time in different experiments (up to 60 min). The initial time course is only given for the extreme values of the Mg concentration range. With the ionophore concentration used in the present experiments, the half time to reach equilibrium was about 2.5 min.

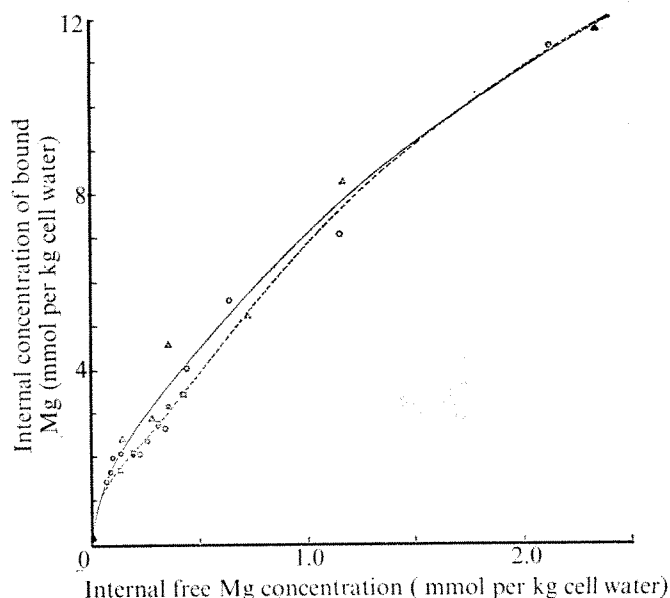


Fig. 2 Equilibrium concentration of bound Mg as a function of the free Mg concentration in cell water. Mg^B was calculated from equation (3) and using the values of Mg^T , r and f shown on Fig. 1. Different symbols correspond to different experiments. The curves correspond to the theoretical equation $Mg^B = C_0 + C_1 (Mg_i^{2+} / (K_1 + Mg_i^{2+})) + C_2 (Mg_i^{2+} / (K_2 + Mg_i^{2+}))^n$ with C and K , in mmol per kg cell water; n is dimensionless. —, $C_0 = 0.1$; $C_1 = 1.5$; $C_2 = 28$; $K_1 = 0.04$; $K_2 = 4$ and $n = 1$; ---, $C_0 = 0.1$; $C_1 = 1.8$; $C_2 = 20.5$; $K_1 = 0.03$; $K_2 = 1$ and $n = 2$.

original cells was estimated at ~ 0.4 mM which is only slightly lower than that measured with a Mg electrode in a stroma-free, freeze-thaw lysate of oxygenated red cells (0.5 mM (ref. 3)).

At present we can only speculate about the nature of the different buffers. The minute fraction of tightly bound, acid-labile Mg may be associated with cytoplasmic components or with the membrane. Analysis of membrane fragments, however, may prove misleading because this binding can be affected by the separation procedure. It is interesting to note that these binding sites cannot be accessible to Ca (refs 9, 10).

Berger *et al.*³, using simulated intracellular conditions but a much reduced haemoglobin level, estimated the dissociation constants of the complexes of Mg with ATP and 2,3 bisphosphoglycerate (DPG) as 83 μ M and 1.67 mM respectively. It is tempting to equate the low and high affinity buffers reported in this study with DPG and ATP respectively. It is difficult, however, to account for the high capacity of the low affinity buffer even in inosine-fed cells, where the levels of DPG are high^{16,17}, unless other intermediates of the glycolytic pathway or haemoglobin, which did not bind Mg *in vitro*³, are also involved. In oxygenated cells, little ATP or DPG is bound to haemoglobin so that displacement of Mg buffers from haemoglobin by high Mg concentrations would be minimal; however a progressive decrease in the internal magnesium activity coefficient at high internal Mg would have the effect of reducing the calculated amount of low affinity buffer and this possibility cannot be ruled out at present.

The method described here can be used to investigate the effect of metabolism and deoxygenation on the Mg buffer curve in intact cells as well as the Mg dependence of active Na and Ca transport.

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Transient pressure changes in Donnan systems displaced from equilibrium

PRESSURE gradients at interfaces of porous membranes can be caused by two factors: (1) discrete changes in concentrations of particles which are excluded from the aqueous pores because of their size, and (2) electrical stresses which are caused by the action of an electric field on the charged particles at the membrane-solution boundary (A.I., theoretical results to be published elsewhere). Only the first factor is important in neutral pore-containing membranes;

the second operates at the boundary of charged pores, that is, in ion exchange membranes.

One of the consequences of theoretical work (A.I., unpublished) applies to Donnan systems. A Donnan system comprises a membrane interposed between two electrolytic solutions one of which contains impermeant polyelectrolyte particles. At equilibrium, the pressure developed across the membrane equals the difference between the osmotic values of the two solutions. The latter is greater than RTC_i , where C_i is the concentration of the impermeant polyelectrolyte on one side of the membrane^{1,2}, and R and T have their usual meanings. The higher pressure develops in the system because of the increase in concentration of the impermeant polyelectrolyte particles at the membrane solution interface, brought about by the presence of an electric potential profile at that region. Solution of the Poisson-Boltzmann equations for the interfacial region (see, for example, ref. 3) shows that, at equilibrium, the concentration of the impermeant particles at the pore-solution boundary, C_i^b , is such that RTC_i^b is equal to the difference between the osmotic values of two solutions.

Since the extent of accumulation of the polyelectrolyte at the membrane-solution boundary is a function of the salt concentration in the same solution, theoretical considerations lead to the following peculiar prediction: addition of salt to the side of the membrane which contains the polyelectrolyte would cause an immediate decrease in the pressure difference between the two sides of the membrane, even though the salt addition led to an immediate increase in the difference between osmotic values of the two solutions. Thus, phenomenologically the system would behave as if the reflection coefficient for the salt were negative. The experiments described confirm that prediction.

The diffusion cell (Fig. 1) consisted of two lucite chambers held in a metal frame. The flat outer margins of each chamber were cast with a Teflon lining and the membrane between the two chambers was sealed by being pressed on

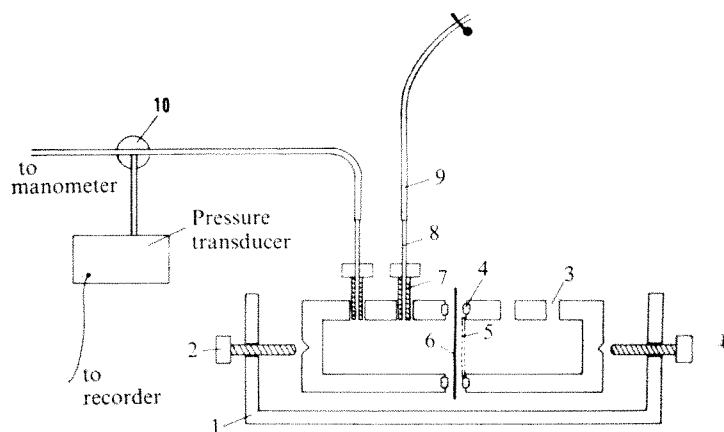


Fig. 1 The diffusion cell. 1, Metal frame; 2, fastening screw; 3, open to room pressure; 4, Teflon lining; 5, stainless steel support; 6, membrane separating the two chambers; 7, plastic screws; 8, syringe needles through the centre of the screws (7); 9, heat-shrinkable Teflon tube; 10, three-way stopcock.

its outer circumference by the Teflon O-shaped linings. On the side of the membrane with the lower pressure, a perforated stainless-steel support (0.5 mm thick) was added. Forty per cent of the support area was perforated. One chamber of the diffusion cell (left in Fig. 1) was sealed as follows: two openings in the upper part of the cell were locked by plastic screws and, to ensure hermetic sealing, the outer rim of the screws were lubricated by high-vacuum silicon grease. Through the centres of these screws, syringe

needles were tightly secured into the chamber. The other end of the needle was connected to a heat-shrinkable Teflon tube. The solution was introduced into the chamber through one of the Teflon tubes. Manipulations of the chamber ensured complete filling of the chamber with the solution. After filling, one Teflon tube was connected to the pressure transducer and the other was clamped. The other chamber (right in Fig. 1) was open to room pressure and was directly filled with the desired solution. Mixing in both chambers was effected by magnetic stirrers and the experiments were conducted at room temperature (24–26 °C). A Statham pressure transducer (Model P23AC) and a Beckman recorder (Type R dynograph) were used. The time constant of the response of the transducer to pressure changes was instantaneous compared with that of the whole diffusion cell system, which was about 3 min.

Analytical procedures included chloride analysis by titration with mercuric nitrate using phenylcarbazone as an indicator, and sodium analysis by flame photometry. Several combinations of membranes and polyelectrolyte were tried. The characteristics required of the membrane were: relatively long aqueous pores, freely permeable to small ions, but completely impermeable to the polyelectrolyte. It was important to use only symmetric membranes. Membranes in which only a thin skin was the selective barrier were obviously unsuitable for this study. The best combination that we found were porous vinyl membranes (Nalco Chemical Company, Chicago) and a sulphonated dextran, of molecular weight of about 500,000 (Sigma Chemical Company, Saint Louis). The thickness of the membranes was $\sim 100 \mu\text{m}$ and the estimated pore diameter about $60 \text{ \AA}$.

The equilibrium pressure characteristics of the system are shown in Fig. 2. The calculated osmotic values of the dextran-containing solution as a function of the chloride

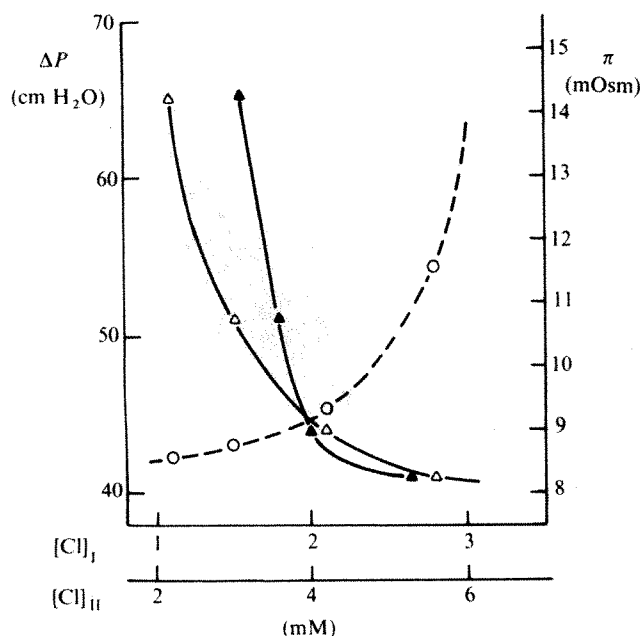


Fig. 2 The equilibrium properties of the diffusion cell when side I of the cell contained sulphonated dextran 9 mg ml^{-1} ; side II contained NaCl only. ---, Calculated osmotic value of the dextran solution as function of chloride concentration $[\text{Cl}]$. The osmotic value of side I was equal to that of side II plus $\Delta P/RT$. The osmotic value of side II was determined by multiplying the chloride concentration by 2 and by the activity coefficient of NaCl at the relevant concentration. The polyelectrolyte solution without chloride contained about 0.018 mM sulphonated dextran and 42 mM sodium. The fact that the osmotic value of the dextran solution is $\sim 8 \text{ mOsm}$ indicates that activity coefficient for Na is ~ 0.2 . Δ , $\Delta P [\text{Cl}]_I$; $\blacktriangle$, $\Delta P [\text{Cl}]_{II}$.

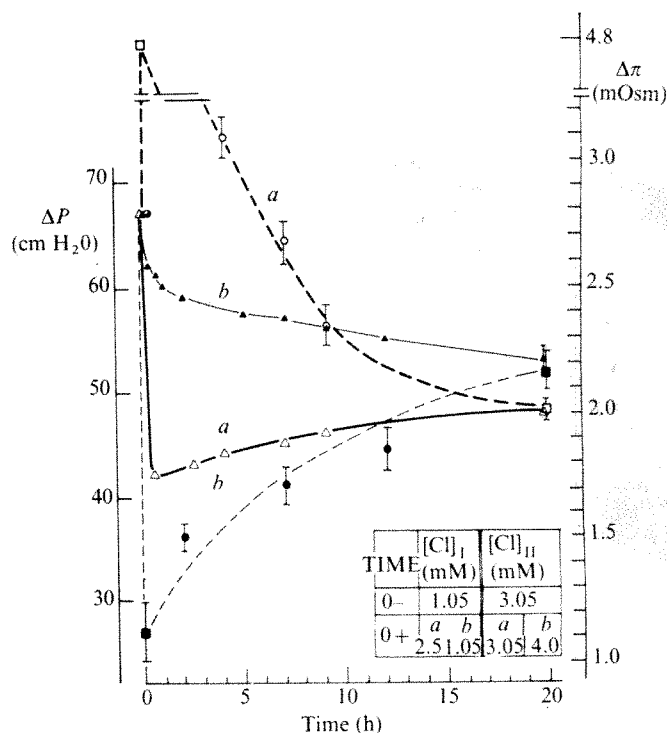


Fig. 3 Time course of change in pressure (Δ , $\blacktriangle$) in a Donnan system brought about by the addition of salt to either side of the cell. *a*, Addition to the sulphonated dextran solution; *b*, addition to the salt solution. Inset, Type of change that was effected at time zero (0_+). The initial conditions (0_-) were the same in both cases. ---, Difference in osmotic values of the two solutions as determined by the chloride concentration in both sides, $\square$, $\blacksquare$, derived by direct chloride analysis; $\circ$, $\bullet$, osmotic values based on calculations of chloride concentrations. $[\text{Cl}]_I$ was estimated from the concomitant ΔP measurements making use of the relevant curve in Fig. 2; $[\text{Cl}]_{II}$ followed from the principle of conservation of matter. The vertical lines represent upper and lower limits which resulted from uncertainties in chloride analysis ($\pm 0.1 \text{ mM}$) and in ΔP measurement ($\pm 0.5 \text{ cm H}_2\text{O}$).

content are also shown in Fig. 2. Chloride was added to the dextran solution as NaCl. The pressure changes accompanying NaCl addition to either side of the system are shown in Fig. 3.

The dashed lines in Fig. 3 represent the difference between osmotic values of the solutions on the two sides of the membrane and curves *a* show that the addition of NaCl to the polyelectrolyte solution lead to increase in the difference between the osmotic values of the two solutions and to decrease in the pressure difference across the membrane.

The results of Fig. 3 could also be accounted for by the assumption that a significant unstirred layer occurs in the side of the membrane which had the metal support. To account for the results along these lines, however, needs an assumption that Donnan equilibrium was attained instantaneously across the membrane and that the diffusion barrier was located only on the side which did not contain the sulphonated dextran. Therefore we have conducted NaCl diffusion experiments through the system with and without the perforated metal support. These experiments demonstrated that the permeability of the membrane to NaCl was 20–30% greater when the metal support was used, probably because the effective membrane area was a little more than the porous area of the metal support. At any rate, these experiments proved clearly that unstirred layers could not constitute a significant barrier in the diffusion cell. The membrane permeability to NaCl was $\sim 3 \times 10^{-4} \text{ cm s}^{-1}$. This level of permeability would correspond to unstirred layer of about $330 \mu\text{m}$ thickness. Analysis of thickness of unstirred layers as a function of stirring rates shows that

they vary from $\sim 75 \mu\text{m}$ at slow rate of stirring to $50 \mu\text{m}$ at vigorous stirring⁴. Thus, it is unlikely that an unstirred layer had a major effect in the system described here.

We suggest that these experiments support the notion (A.I., unpublished) that the pressure across the porous membrane is determined by the concentration of the impermeant polyelectrolyte particles at the membrane solution interface. The extent of accumulation of the polyelectrolyte molecules at the membrane interface is a function of the salt concentration in the solution. At equilibrium, that is, in classical Donnan systems, the accumulation of the polyelectrolyte molecules at the membrane interface gives rise to a pressure difference which is exactly equal to the difference between the osmotic values of the two solutions.

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Functional striated muscle cells from non-myoblast precursors following 5-azacytidine treatment

5-AZACYTIDINE (aza-CR)—a *s*-triazine nucleoside analogue of cytidine—has strong inhibitory effects in various biological systems including neoplasms: it is a powerful bacteriostatic, antitumour and mutagenic agent¹. Aza-CR also exhibits immunosuppressive, antimitotic, radioprotective and virostatic effects¹. The cytostatic effect of aza-CR has primarily been directed against leukaemia² and it has been used to treat human solid tumours³. During a study on the morphological transformation of C3H 10T $\frac{1}{2}$ C18 cells by cancer chemotherapeutic agents, it was noted that elongated multinucleated cells arose in cultures exposed to aza-CR⁴. The C3H 10T $\frac{1}{2}$ C18 line is a clonal line of mouse embryo cells developed at the McArdle Laboratory for Cancer Research for use in studies on chemical oncogenesis *in vitro*⁵⁻¹⁰. The cells are highly sensitive to post-confluence inhibition of cell division, show a remarkably low rate of spontaneous transformation and grow with a fibroblast-like morphology with long cytoplasmic processes in sparse cultures. When confluent, they form flat even monolayers and appear epithelioid. Scanning electron microscopy has shown the confluent cells to be extremely thin and polygonal with smooth surfaces resembling epithelial cells¹¹. Here we describe experiments showing that the multinucleated tubular cells which arise in C3H 10T $\frac{1}{2}$ C18 cells treated with aza-CR are in fact functional myotubes.

The formation of a large focus of multinucleated cells in a culture of C3H 10T $\frac{1}{2}$ C18 cells treated with aza-CR is shown in Fig. 1a. The long tubular characteristics of the cells growing on a monolayer of mononucleated cells should particularly be noted. These tubular cells contained many nuclei with distinct nucleoli (Fig. 1b). The background of C3H 10T $\frac{1}{2}$ C18 cells can also be seen.

In some such cells, regular striations were apparent (Fig. 1c), with a repeating unit length of $2.7 \mu\text{m}$. The striations were a feature which appeared shortly after the formation of the multinucleated cells about 9-10 d after treatment with aza-CR. Although the multinucleated cells sometimes persisted for up to 20 d after their appearance, most of them started to atrophy soon after they were

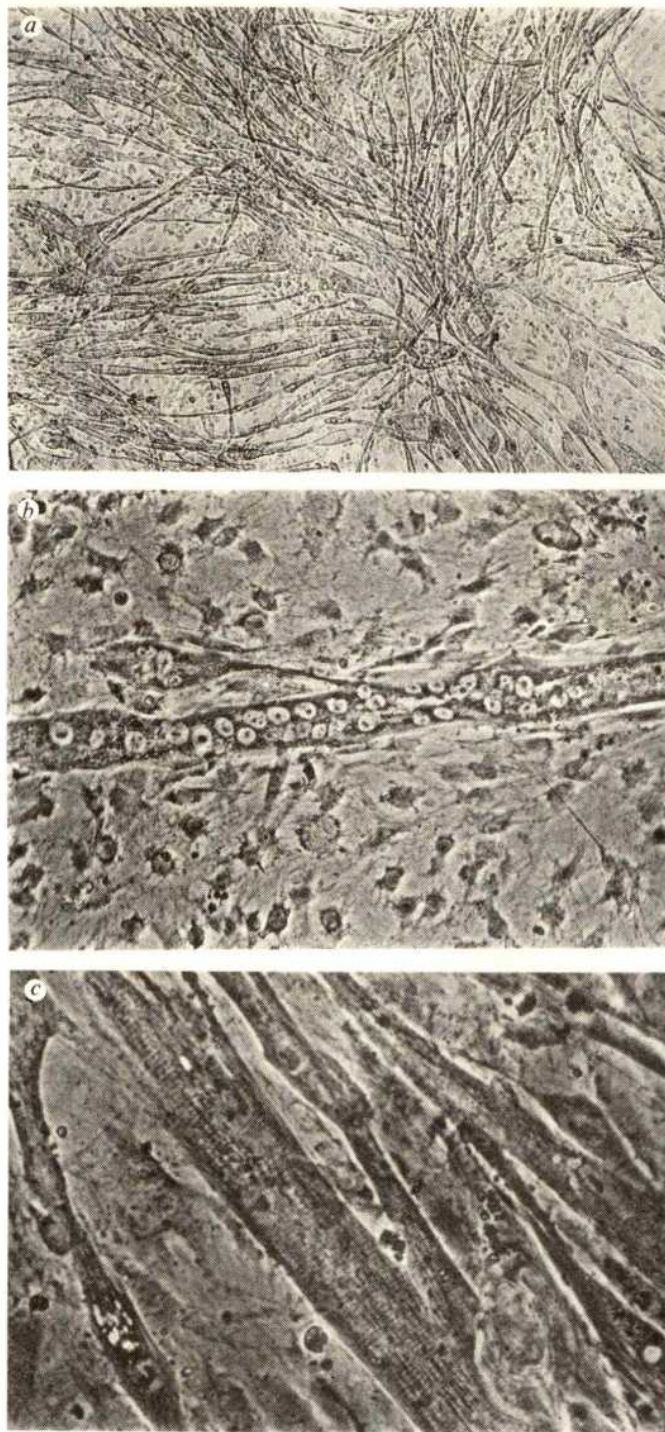


Fig. 1 Myotubes induced in C3H 10T $\frac{1}{2}$ C18 cells by aza-CR. a, Low magnification ($\times 69$) of a living culture showing a focus of multinucleated cells. b, Phase contrast microscopy of a living culture showing many nuclei with distinct nucleoli in a multinucleated cell. The background of evenly-distributed nuclei of the C3H 10T $\frac{1}{2}$ C18 monolayer can also be seen ($\times 252$). c, Phase contrast microscopy of a living culture showing clearly defined, regular striations ($\times 546$).

formed and not many remained longer than 14 d. Table 1 lists some of the physical characteristics of these cells. They are macroscopic and many can be seen with the naked eye. They are generally long and thin, being on average 20 times longer than they are thick. The tubular cells are found either isolated or in foci (for example, Fig. 1a); however, the majority of multinucleated cells are found in foci. There is a large variation in the number of nuclei per cell, ranging from 2 to approximately 2,000, the average being 8.6 nuclei per cell.

A small percentage of the multinucleated cells have occasionally been seen to contract spontaneously or twitch in culture about 7 d after their appearance. This twitching continued for various lengths of time, between 1 and 7 d depending on the stage of atrophy of the cells. Twitching was generally seen in the middle or late atrophy stages of

We consider these cells which arise in aza-CR treated cultures to be muscle cells for the following reasons: (1) The cells are multinucleated and tubular in morphology. (2) Striations are evenly and regularly spaced and found to be 2.7 μ m long. (3) Spontaneous contractions of the cells have been seen in several experiments. (4) The cells

Table 1 Physical characteristics of multinucleated tubular cells arising in aza-CR-treated cultures

Character	Average value	Range	No. of cells examined
Length	0.9 mm	0.16–3.96 mm	23
Width	0.043 mm	0.007–0.37 mm	23
No. of nuclei per cell	8.6	2–2,000	736
Twitching rate	88 contractions per min	55–160 contractions per min	14

The measurements were made on fixed and stained dishes with the exception of the twitching rate which was determined in living cultures.

the life of multinucleated cells, and the twitching rate shown in Table 1 varied from 55 to 160 contractions per min. The twitching was generally localised to small areas of the cells, although waves of contraction have been seen to pass through a length of a focus in one case.

Cells possessing striations have been artificially stimulated to contract with acetylcholine. This was done by placing a drop of 1 mM acetylcholine over the cell in question while observing it with the inverted microscope. This technique was not successful in every instance but some tubular cells contracted strongly and then remained in the contracted state.

The effect of aza-CR was markedly dependent on the concentration of analogue used (Table 2). Little cytotoxicity, as measured by decrease in plating efficiency, was seen with a level of 3.0×10^{-7} M aza-CR and a few multinucleated cells were formed. A concentration of 10^{-6} M aza-CR, however, caused appreciable cell death while there was a very large increase in the number of nuclei appearing in multinucleated cells. A dose of 3.0×10^{-6} M aza-CR was considerably more cytotoxic (80% decrease in plating efficiency) and fewer nuclei appeared in tubular cells. But, when a correction was made for the extent of cell killing, this dose was also effective in inducing the formation of multinucleated cells. Table 2 also shows that the intact triazine ring of aza-CR is necessary for this effect. The ring is rapidly hydrolysed¹² at slightly alkaline pH and we found the half-life of aza-CR to be about 90 min at 50 °C in phosphate-buffered saline at pH 7.4. When the stock solution was kept at this temperature for 20 h it lost its ability to cause cytotoxicity and multinucleation. Finally, it should be noted that there are approximately 600,000 mononucleated cells in the dishes at confluence so that the percentage of nuclei appearing in tubular cells is relatively small even under optimum conditions.

respond positively to acetylcholine which points to the presence of functional acetylcholine receptors. None of these properties has been found in untreated C3H 10T $\frac{1}{2}$ C18 cultures or in cultures treated with many other agents. Furthermore there is no other evidence to suggest that the line is myoblastic in nature^{5,11}. It is unlikely that aza-CR selects for possible myoblast contaminants in the parent line since these effects have been found in a laboratory where myoblasts were not grown⁴ and multinucleated tubular cells are never seen in untreated controls. An additional point is that the multinucleated cells were induced in two separate batches of C3H 10T $\frac{1}{2}$ C18 cells which were obtained from the McArdle laboratory. The effects of aza-CR are reproducible although we have found variations in the absolute numbers of fused nuclei in different experiments.

We therefore conclude that the production of muscle cells was induced by aza-CR. Piled-up foci of other cell types were also formed following treatment with aza-CR. We are currently attempting to characterise them, since it is possible that other types of phenotypic conversion may also occur.

Two possibilities for the origin of the multinucleated cells are (1) endoreduplication and (2) fusion of many mononucleated cells. Preliminary photographic experiments have shown many bipolar mononucleated cells in areas where multinucleated cells subsequently appeared suggesting that fusion was occurring. It seems, therefore, that aza-CR causes regulatory changes in the C3H 10T $\frac{1}{2}$ C18 cells which leads to some of them becoming myoblasts. The precise cell type from which the C3H 10T $\frac{1}{2}$ C18 cell is derived is not known, but this information will be of value in assessing the extent and the nature of the change induced by aza-CR. It seems possible, however, that the compound will be of value in studying differentiation in an analogous way to the use of

Table 2 Formation of multinucleated cells after treatment of C3H 10T $\frac{1}{2}$ C18 cells with aza-CR

Concentration of aza-CR (M)	Plating efficiency (%)	Average no. of nuclei in multinucleated tubular cells* per dish	No. of nuclei in multinucleated cells per 100 cells surviving treatment
0	25	0	0
3.0×10^{-7}	22	133	60
1×10^{-6}	13	4,624	3,557
3.0×10^{-6}	5	1,637	3,274
1×10^{-6} , preheated at 50 °C for 20 h	26	0	0

Stock solutions of aza-CR were made up fresh for each experiment in phosphate-buffered saline pH 7.4, sterilised by filtration and kept at 0 °C until used. C3H 10T $\frac{1}{2}$ C18 cells were used in the 15–20th passage and were grown in Eagle's basal medium supplemented with 10% heat-inactivated foetal calf serum and penicillin and streptomycin. Cells obtained from log-phase mass culture were seeded into 60-mm dishes (1,000 cells per dish) and treated 24 h later with the indicated doses of aza-CR. Exposure was for 24 h, after which the cultures were washed and maintained for 14–16 d. The dishes were then rinsed with water, fixed with methanol and stained with May/Grünwald and then Giemsa stains. The dishes (four per group) were then scored for nuclei in multinucleated cells at 100 $\times$ magnification. Cell killing was determined by the lowering of plating efficiency in similarly treated cultures containing 200 cells and stained with Giemsa stain after 10–12 d. Data given are the average values from two separate experiments.

*A multinucleated tubular cell was defined as an elongated cell having two or more nuclei.

5-bromo-2'-deoxyuridine¹³. In this regard it is of obvious interest that the analogue is incorporated into nucleic acids with a high efficiency¹⁴.

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Voltage-dependent effect of curare at the frog neuromuscular junction

CONDUCTANCE measurements on various cholinergic post-junctional membranes have demonstrated voltage-dependent effects for acetylcholine (ACh) and its agonists¹⁻⁵, for local anaesthetics⁶, and for curare⁵. It is not yet clear which of the steps in drug-receptor interaction are rate-limiting for voltage-dependent effects of ACh. Kordaš⁷ considers this step to be the

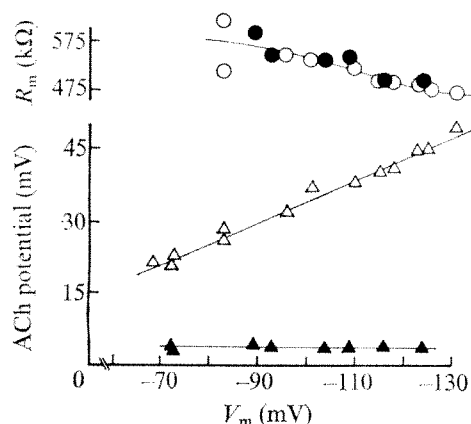


Fig 1 Lower: Peak amplitude of the ACh potential (ordinate) plotted against the membrane potential (V_m , abscissa) for data obtained during the control period (Δ) and then, several minutes later, in the presence of $4.3 \mu\text{M}$ TC-Ringer ($\blacktriangle$). The membrane potential plotted is that value measured just before the beginning of an ACh potential. The amount of ACh released by iontophoresis was constant throughout, the total charge released per iontophoretic pulse being 4.37 nC . The data were obtained while the membrane was hyperpolarised away from and, then, repolarised back to the resting potential. Note that the ACh potential amplitude increased with hyperpolarisation while the preparation was bathed in normal Ringer but that it remained essentially unchanged during hyperpolarisation when curare was present. Upper: The input resistance of the muscle fibre (ordinate) is plotted against membrane potential. Measurements made before ($\circ$) and in the presence of curare ($\bullet$) show that the small degree of anomalous rectification¹³ observed in this fibre was unaffected by curare.

initial binding between ACh and its receptor, whereas Magleby and Stevens⁸ consider the conformational changes between open and closed states of the receptor to be rate-limiting. Distinct from the initial binding reaction and the subsequent conformational change is the possibility of a drug binding to a receptor that is in its open, rather than closed, state, thereby forming a complex consisting of three molecular species. This idea comes from Steinbach's model⁹ for the action of local anaesthetics at the frog endplate. Such multi-molecular complexes have been included in reaction schemes which describe voltage-dependent effects for local anaesthetics at the frog endplate¹⁰ and for curare in *Aplysia* neurones⁵. Marty *et al.*⁵ found that during hyperpolarisation of molluscan neurones, curare became a more effective blocker of the response to slowly applied ACh. Such an effect of curare was not seen when ACh was applied quickly to the receptors, that is when there was insufficient time for ACh to equilibrate with its postsynaptic effect. They suggested that the binding between curare and the cholinergic receptor is

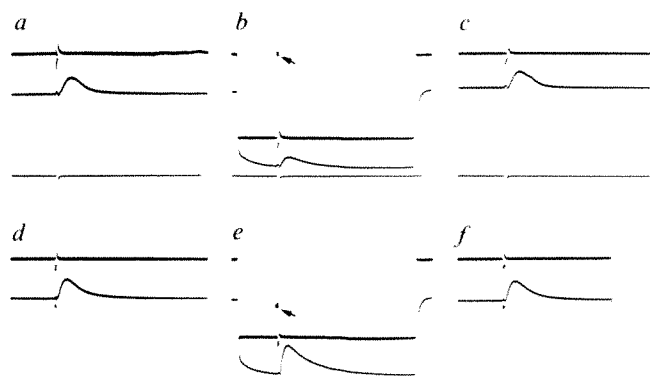


Fig. 2 Reversibility of the increased blocking effect of curare during hyperpolarisation. Six oscillograms (a-f) were obtained sequentially from the same endplate. Three CRO traces are present in each oscillogram. Upper trace, hyperpolarising current (inward current, downward) applied to the fibre from an intracellular microelectrode. Middle trace, intracellular recording of membrane potential. Lower trace, iontophoretic current (outward current, upward) through the ACh pipette. (Arrows point to the tops of the ACh current pulses in b and e.) a-c: ($4.3 \mu\text{M}$ TC present) A response was first obtained at the resting potential (a). The ACh potential decreased during hyperpolarisation (b) and returned to its control value when ACh was applied again at the resting potential (c). The preparation was then washed with curare-free Ringer, and about 2 min later, the recordings in d-f were obtained. The amount of ACh released by iontophoresis, held constant in a-c, was decreased until the amplitude of the ACh potential in d was about equal to that in a. The ACh potential increased with hyperpolarisation (e). The resting potential was -63.4 mV . Total charge released from ACh pipette: a-c, 18.0 nC ; d-f, 5.74 nC . Horizontal calibration: 400 ms . Vertical calibration: 100 nA (membrane current); 40 mV (V_m); 200 nA (iontophoretic current).

voltage-dependent but that this particular kind of binding can only occur between curare and a receptor which is already in its open state. Here I show that curare has a very similar effect at the frog neuromuscular junction. The efficacy of curare as a postsynaptic blocker at the frog neuromuscular junction increases when the muscle fibre is hyperpolarised. This effect is seen only when ACh is applied slowly, however, and not when it is applied rapidly during neural release of ACh.

Experiments were performed at room temperature ($19-23^\circ\text{C}$) on sartorius muscles from *Rana pipiens*; in some experiments, nerve muscle preparations were used. The composition of the normal Ringer (mM) was: 111 NaCl ; 2.5 KCl ; 2.0 CaCl_2 ; $0.4 \text{ NaH}_2\text{PO}_4$; $1.1 \text{ Na}_2\text{HPO}_4$ (pH 6.9). Conventional procedures were used for applying ACh by iontophoresis^{11,12}, for recording membrane potential with an intracellular microelectrode (containing 1 M potassium citrate), and for changing membrane potential by passing constant current pulses through a second intracellular microelectrode. The ACh pipettes had tip diameters

of about 1 μm and electrical resistances of 30–60 M Ω . The oscillograms shown in Fig. 2 are representative of the duration of the constant current pulses used to change the membrane potential and the time course of the responses to iontophoretically applied ACh (ACh potentials). ACh was applied at 15-s intervals. The responses to iontophoretically applied ACh were always much slower (by a factor of about 50–100) than were those due to ACh released from nerve terminals.

Figure 1 shows an experiment in which the amplitude of the ACh potential was measured at various membrane potentials with and without 4.3 μM tubocurarine (TC). In the absence of curare the ACh potential increased together with the driving force as the muscle fibre was hyperpolarised. In the presence of curare, however, the ACh potential, quite unexpectedly, did not increase with hyperpolarisation. This phenomenon is reversible in two respects. First, the increased action of curare was only seen during hyperpolarisation (Fig. 2b); it was not seen when the hyperpolarising pulse was no longer applied (Fig. 2c). Second, when the curare was removed by washing with curare-free Ringer (Fig. 2d–f), the phenomenon was no longer observed (Fig. 2e).

The voltage-dependent effect of curare shown in Figs 1 and 2 is not simply an apparent effect resulting from errors inherent in the measurement of postsynaptic potentials due to the non-linear electrical characteristics of the muscle fibre¹³ and/or to the fact that the instantaneous driving force actually decreases during a postsynaptic potential. Previous work¹⁴ and data from this study (Figs 1 and 2 b, e) show that curare does not affect the electrical properties of the nonsynaptic membrane. In addition, when the iontophoretic current was adjusted so that ACh potentials of almost equal amplitudes were obtained at the resting potential both in the presence and absence of curare (Fig. 2 a, d), the ACh potential decreased during hyperpolarisation only when curare was present (Fig. 2b).

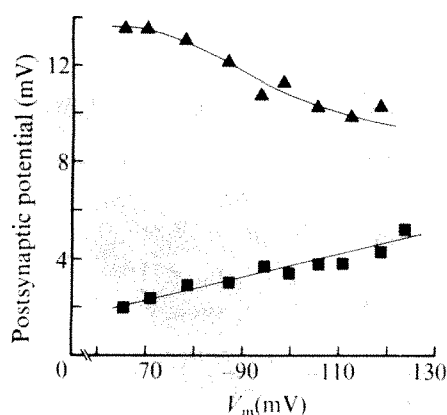
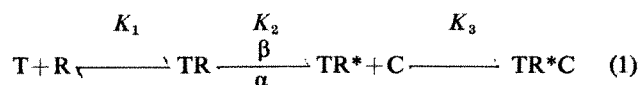


Fig. 3 Comparison of the effects of hyperpolarisation on the ACh potential and e.p.p. in the presence of curare. The amplitudes of the postsynaptic potentials due to the iontophoresis of ACh (ACh potentials, $\blacktriangle$) and to the synchronous release of ACh quanta from nerve terminals (e.p.p.s, $\blacksquare$) are plotted on the ordinate against membrane potential (abscissa). All data are from the same endplate. 3.6 μM TC was present. The ACh potentials and the e.p.p.s were recorded on alternate sweeps as the muscle fibre was hyperpolarised. Either the nerve was stimulated or ACh was applied by iontophoresis approximately 400 ms after the start of a hyperpolarising pulse. The e.p.p. increased with hyperpolarisation, as expected, while the ACh potential decreased. The increased action of curare with hyperpolarisation was not seen with neurally released ACh; this action was only observed when ACh was applied more slowly to the receptors by iontophoresis. In this experiment, the input resistance decreased from 800 k Ω during minimal hyperpolarisation to 400 k Ω during maximal hyperpolarisation. Therefore, there is a possibility that, due to the complex electrical properties of the muscle fibre^{9,17}, anomalous rectification influenced only the slow ACh potential and not the fast e.p.p.

The simplest model to account for this voltage-dependent effect of curare would involve an increased binding between curare and the closed cholinergic receptor during hyperpolarisation. But, if that were true, then the response to ACh released from nerve terminals should also reflect this increased binding of curare. The work of Takeuchi and Takeuchi¹⁵, and data in Fig. 3 show that such a voltage-dependent effect of curare is absent in experiments dealing with the neural release of ACh. In the experiment shown in Fig. 3, simultaneous recordings were made of the endplate potential (e.p.p.; neural release of ACh) and the ACh potential (iontophoresis of ACh). During hyperpolarisation the e.p.p. increased in a manner similar to that seen for neurally evoked endplate currents¹⁵, while the ACh potential decreased.

Thus, we can reject the possibility that curare binds more effectively to the closed receptors during hyperpolarisation, and conclude that there must be two mechanisms for the action of curare. One mechanism is dependent on membrane potential and the other is not. The first mechanism is the classical one¹⁶ in which curare binds to the closed cholinergic receptor and competes with ACh for binding sites; the classical action of curare is voltage-independent. The second mechanism, described in this study (see also ref. 5), might involve curare binding to the ACh receptor after it has already undergone a conformational change. Since an ACh receptor undergoes a conformational change during its interaction with ACh, I suggest that it is the activated ACh receptor complex⁹ (open state of the receptor) to which curare can bind with increasing effectiveness during hyperpolarisation. The proposed reaction scheme, which is based on that given by Ruff¹⁰ for the effect of a local anaesthetic on ACh-receptor-interaction, is



where K_1 represents the initial binding between agonist and receptor, K_2 represents the conformational change to an open receptor (TR^*), and K_3 represents the voltage-dependent binding reaction between the open receptor and curare to form TR^*C . It is assumed that the conductance of TR^*C would be zero.

Neural release of ACh is so fast that ACh is not in an equilibrium with its postsynaptic effect, whereas such an equilibrium can be attained when ACh is slowly applied by iontophoresis^{1,18,19}. It is of interest to point out a similarity between the voltage-dependent effect for ACh (that in which the ACh-induced conductance increases with hyperpolarisation^{1–5,20}) and that for curare. Both effects are observed only under equilibrium conditions, that is, both are absent when ACh is released quickly from nerve terminals (Fig. 3; refs 1 and 15). One may then ask if similar mechanisms are involved. Thus, can an activated agonist-receptor complex bind slowly not only to local anaesthetics and to antagonists, but also to agonists?

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Immune lesions of noradrenergic neurones in rat central nervous system produced by antibodies to dopamine- β -hydroxylase

THE development of antibodies against several neurotransmitter synthesising enzymes has facilitated immunohistochemical identification of specific neurones in the nervous system^{1,2}. The availability of these antibodies has also raised the possibility of producing immune lesions of the neurones in situations where the antibody has access to its antigen *in vivo*. In the case of synaptic vesicle antigens, the vesicle membrane becomes incorporated into the nerve terminal membrane during release of transmitter by exocytosis³⁻⁵. Dopamine- β -hydroxylase (DBH), the enzyme that synthesises noradrenaline, is located in the membrane of noradrenergic synaptic vesicles and would become exposed to antibodies in the extracellular space during nerve activity⁶. Binding of injected DBH antibodies has been demonstrated both histochemically and biochemically in sympathetic nerves⁷⁻⁹. Antibodies to DBH cause degeneration of noradrenergic nerve terminals in several guinea pig tissues when injected intravenously^{10,11}; this form of immunosympathectomy seems to be a complement-mediated reaction¹². We report here that injection of anti-DBH serum and complement into the cerebrospinal fluid (CSF) of rats produces degeneration of noradrenergic nerve fibres in the central nervous system.

Female Porton rats (200-250 g) were anaesthetised with intraperitoneal sodium pentobarbital (42 mg per kg body weight) and placed in a Kopf stereotaxic apparatus. A 21 or 23 gauge steel needle connected to a Harvard infusion pump was inserted stereotactically in either a lateral or the third ventricle. Rabbit anti-bovine DBH serum¹³ (50-250 μ l) mixed with complement (50-250 μ l of guinea pig serum) was infused over 1-2 h. Outflow of CSF and antiserum occurred from the subarachnoid space around the infusion site or from a cannula inserted in the cisterna magna. Nine rats received anti-DBH serum and complement and four control rats received equivalent volumes of non-immune rabbit serum and complement.

At times varying from 2 to 7 d after antibody infusion, rats were anaesthetised, a cannula inserted retrogradely into the abdominal aorta and the right atrium was cut. Perfusion was started with normal saline and continued for 1 h with 4% formaldehyde and 0.5% glutaraldehyde (electron microscopy grade) in 0.1 M sodium phosphate buffer, pH 7.0. This aldehyde mixture has recently been shown to fix the nervous system and simultaneously convert catecholamines to fluorescent derivatives¹⁴. The brain and upper spinal cord were removed, cut into 5 mm transverse slices, and 30- μ m sections cut on a Vibratome at 4 °C. The freshly cut sections were photographed at low magnification using transmitted white light and dried over phosphorus pentoxide for 1 h. The sections were then mounted in liquid paraffin or Fluormount and examined with a Leitz Ortholux II microscope, fitted with a high pressure mercury vapour lamp. Both control and experimental sections were studied immediately after preparation of the sections before fading of the finest terminals. The distribution of fluorescent, catecholamine-containing structures was mapped on prints of the original white light photographs¹⁴.

Many thick and distorted fluorescent axons were visible throughout the brains of all animals that received immune serum (Fig. 1a). They were most numerous in the medial septal nuclei, diagonal band of Broca, piriform cortex,

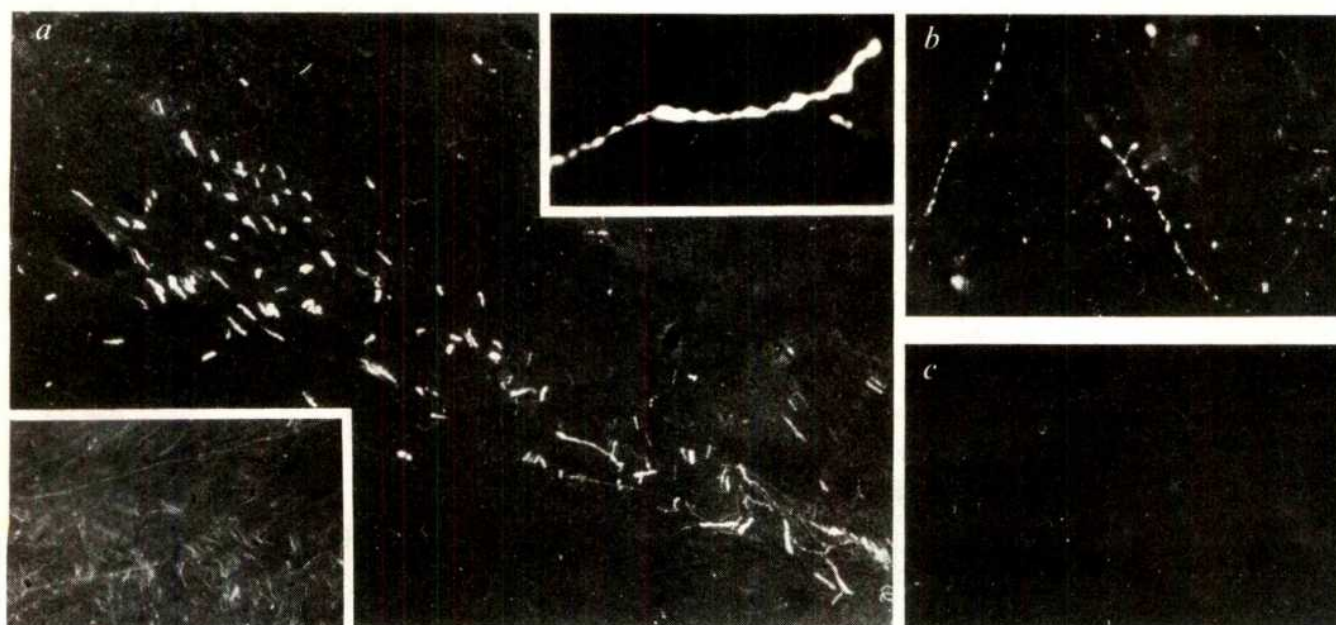


Fig. 1 a, Fluorescence photomicrograph of a transverse section through the zona incerta of a rat brain 3 d after anti-DBH serum and complement were infused into the third ventricle. Many obliquely sectioned noradrenergic fibres are thick and distorted. ($\times 165$.) Insert top right: a single degenerating axon in the same area. ($\times 400$.) Insert lower left: zone incerta of normal rat brain showing oblique sections of bundles of ascending noradrenergic axons. ($\times 165$.) b, Fluorescence photomicrograph of a transverse section through the hippocampus of a rat brain 3 d after non-immune serum and complement were infused into the lateral ventricle. Many normal varicose axon terminals are present. ($\times 330$.) c, A corresponding hippocampal area in a rat 3 d after anti-DBH serum and complement were infused into the lateral ventricle. The noradrenergic terminals are no longer apparent. ($\times 330$.)

rostral corpus callosum, zona incerta, posterior commissure, dorsal tegmental bundle and occasionally in fibre tracts in the lateral funiculi of the thoracic spinal cord. In contrast to the fine smooth appearance of normal catecholamine-containing axons, the degenerating fibres were very brightly fluorescent and irregularly swollen. In longitudinal sections the axons often appeared as a series of bulges connected by fine strands.

In addition to the swollen axon bundles, there was a patchy loss of varicose axon terminals accompanied by occasional swollen varicosities in the neocortex, hippocampus and cerebellar cortex (Fig. 1c). The catecholamine-containing cell bodies in the brain stem seemed normal. In the control rats, there was no detectable loss of varicose axon terminals (Fig. 1b) and the only swollen fibres seen were confined to the area of local necrosis along the pathway of the cannula.

The distribution of the swollen fluorescent fibres occurs within the general distribution of known noradrenergic fibre tracts¹⁵⁻¹⁷, and their appearance resembles that of degenerating noradrenergic axons in the peripheral nervous system induced by antibodies to DBH (ref. 10). The immune lesions are probably produced when antibodies bind to DBH exposed in the axon terminal membrane during exocytotic release of transmitter with subsequent lysis of the terminal membrane by complement fixed by the immune reaction¹². This destruction of axon terminals leads to an accumulation in preterminal axons of noradrenaline-synthesising vesicles transported from the neurone cell body⁶.

The lesions in the central nervous system are likely to be confined to noradrenergic and adrenergic neurones in which DBH is localised. The specificity of the antiserum used has been well defined by both immunochemical⁶ and immunohistochemical procedures¹⁰ and, in the peripheral nervous system, the degeneration produced by intravenously administered anti-DBH was confined to noradrenergic nerves¹². In principle, the use of an antiserum directed against a specific constituent of synaptic vesicle membranes could be extended to produce immune lesions of neurones using transmitters other than noradrenaline.

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Possible role of pH gradient and membrane ATPase in the loading of sucrose into the sieve tubes

FOR plants to grow, sugars and other assimilates must constantly be transported from the leaves to the growing or storage regions. This essential transport function takes place in the highly specialised phloem tissue which consists of a network of interconnecting sieve tubes. In spite of its obvious agronomic importance, little is known about the first step of sugar entry (loading) into the sieve tubes. Here I propose a model which formulates several of the established, but difficult to reconcile, characteristics of the sieve tubes into a unifying model for sugar uptake. These characteristics include the alkaline sap¹ (pH 8-8.5) with a high potassium ion and ATP² concentration, the presence

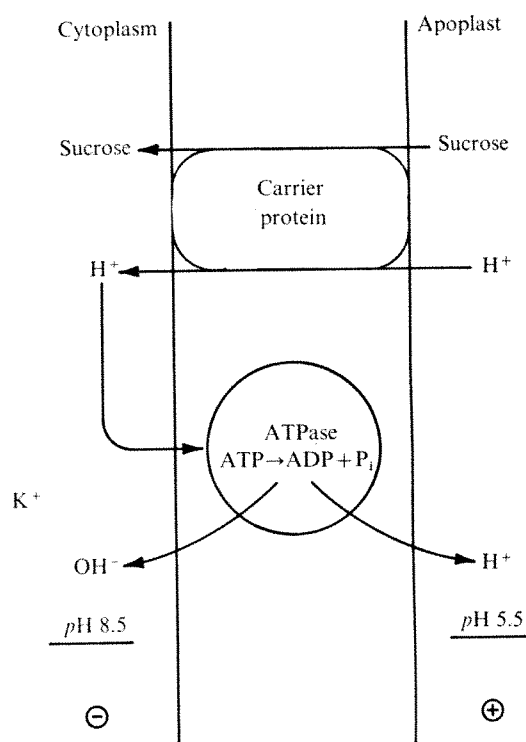


Fig. 1 Model of transport into sieve tubes

of electrical gradients³ and the considerable ATPase⁴ activity of the sieve tube plasmalemma. According to this model, sucrose leaves the site of synthesis in the mesophyll cells and enters the free space or apoplast which has a pH of 5 to 6. The sucrose is then accumulated into the alkaline sieve tubes by an active process involving membrane sulphhydryl groups⁵. In this model the proton gradient of nearly 3 pH units across the sieve tube plasmalemma provides the driving force for sucrose uptake (Fig. 1). Based on non-electrolyte transport studies in bacteria⁶, algae⁷ and fungi⁸ it is proposed that protons are cotransported with sucrose into the sieve tubes.

The proton gradient across the membrane is maintained for continuous sucrose influx by an ATP-driven proton extrusion system wherein ATP is hydrolysed by the plasmalemma ATPase in such a way that an asymmetrical distribution of protons and hydroxyl ions is established across the sieve tube plasmalemma thereby maintaining the internal alkalinity. Studies on the cotransport of hexose and protons in the algae⁷ and fungi⁸ indicate that both a proton gradient and its resulting electrical potential are

involved in uptake. In this regard, it should be noted that potassium ions have been implicated in electrical gradients in the phloem³. Thus, it would be most valuable to establish the magnitude of any electrical potential difference across the sieve tube membrane during sucrose uptake.

Data on phloem loading of sucrose support the model. Using non-permeant buffers to change the pH of the apoplast, sucrose uptake into the sieve tube and translocation from the source leaf of an intact plant were inhibited when the magnitude of the pH difference between the apoplast and symplast was reduced⁹. The pH optimum for phloem loading is 5, and loading is markedly inhibited at pH 8. In addition, the affinity of the carrier for sucrose is decreased at pH 8. Thus, proton and sucrose molecules can bind to a membrane carrier which displays a higher affinity in the protonated form (Fig. 1). The charged complex (sucrose-H⁺-carrier) can be driven across the membrane by the electrical potential difference generated by the proton extrusion mechanism.

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Echovirus 12-induced host cell shutoff is prevented by rhodanine

INFECTION of animal cells with picornaviruses results in inhibition of host cell RNA and protein synthesis shortly after infection, as was shown with members of the cardiovirus group¹⁻³, with poliovirus⁴⁻⁶, and with foot-and-mouth disease virus⁷. The molecular mechanism of this so-called shutoff is not well understood; however, it seems to require protein synthesis early in infection^{2,8,9}, but no viral RNA replication⁹⁻¹¹. We report here that 2-thio-4-oxothiazolidine (rhodanine), at a concentration which inhibits selectively the uncoating of echovirus 12 in GMK cells also inhibits the virus-induced shutoff of GMK cell protein synthesis.

The infection of GMK cells, a permanent cell line derived from African green monkey kidney cells¹², with echovirus 12 (prototype Travis) is followed by inhibition of the GMK cell protein synthesis to the extent of 80% by 3 h post infection (p.i.). A clear peak of viral protein synthesis is visible 4-5 h p.i. RNA synthesis declines more gradually, showing a specific shoulder 4-5 h p.i. due to viral RNA synthesis. At 3 h p.i. viral RNA and protein synthesis is not yet detectable in the conditions of our experiments and formation of new particles has not yet started¹².

Rhodanine acts by blocking uncoating of the virus without inhibiting virus adsorption or penetration¹². Nor does it affect the replication of purified echovirus 12 RNA in GMK cells. Furthermore, infective virus could be recovered quantitatively from infected, rhodanine-treated cells and rhodanine was shown to protect the echo 12 virion from alkaline degradation *in vitro*. Rhodanine apparently acts by stabilising the virion structure. We also expected to find that rhodanine would inhibit the virus-induced shutoff of host cell protein synthesis. Indeed, our experiment showed that no host shutoff takes place in echovirus 12-infected cells in the presence of rhodanine, whereas in the untreated

control host cell protein synthesis is repressed to less than 20% of the mock-infected control by 3 h p.i. (Fig. 1).

In contrast the shutoff induced by poliovirus 2 (strain P 712-ch-2ab) was not affected by rhodanine (Fig. 1). That is consistent with the fact that the multiplication of poliovirus 2, unlike that of echovirus 12, is unaffected by rhodanine^{12,13}.

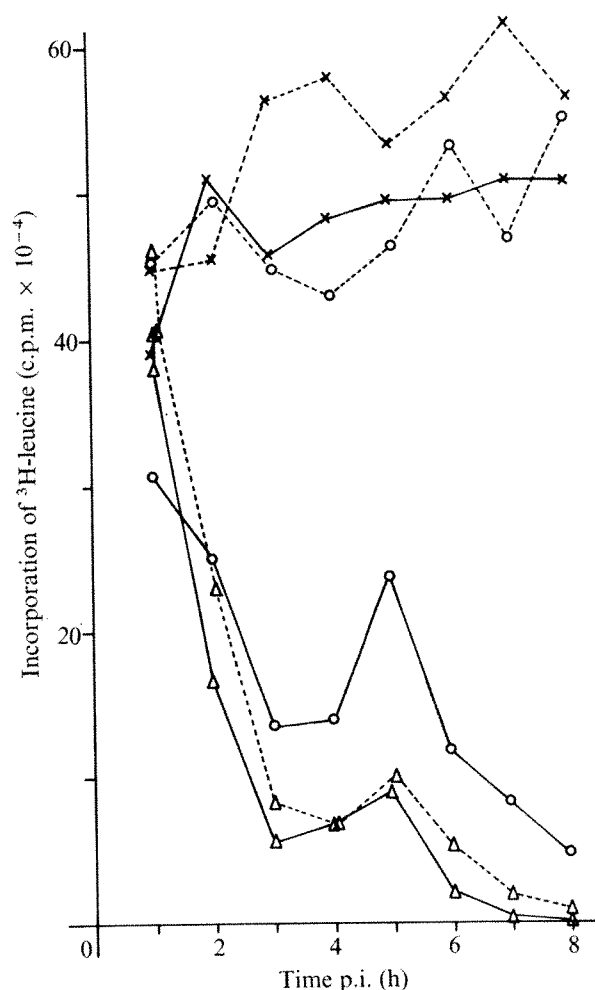


Fig. 1 Confluent monolayers of GMK cells were infected with echovirus 12 (20-50 plaque-forming-units (PFU per cell) or poliovirus 2 (50-100 PFU per cell) in the absence or presence of 100 $\mu\text{g ml}^{-1}$ rhodanine. Rate of protein synthesis was measured as follows: L-(4, 5-³H)-leucine was added to an aliquot of the cells at various times p.i. The cells were collected 30 min later, and the radioactivity incorporated into trichloroacetic acid precipitable material was determined. Broken lines denote presence of rhodanine, solid lines denote absence of rhodanine. $\times$, Mock infection; $\circ$, echovirus 12 infection; Δ , poliovirus 2 infection.

The shutoff effect by echovirus 12 is, within experimental limits, as dependent on the multiplicity of infection as it is with poliovirus 2 (Fig. 1). We do not know whether the effects of rhodanine would persist at still higher multiplicities. This experiment might be complicated by emergence of rhodanine-resistant or -dependent mutants¹².

This result demonstrates that the specific inhibitor of uncoating of echovirus 12, rhodanine, also prevents the virus-induced shutoff of host protein synthesis. Obviously, adsorption and penetration are not sufficient to induce the effect; uncoating is a necessary prerequisite for this shutoff to occur. The virion per se—for example, by depressing some cellular function(s)—is not able to induce host cell shutoff.

Rhodanine thus seems to fulfil some of the ideals

clinicians ask for in viral chemotherapies—it affects an early stage of virus-cell interaction leaving intact vital functions of the cell.

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Guanosine tetra- and pentaphosphate synthesis by bacterial stringent factor and eukaryotic ribosomes

AMINO acid starvation of *rel*⁺ bacteria¹ results in a rapid pleiotropic response involving the cessation of stable RNA synthesis, a decrease in the rate of nucleoside transport, an altered pattern of mRNA synthesis, inhibition of phospholipid synthesis and increased protein turnover. This stringent response² is rapidly reversed when the amino acid starvation is relieved and is apparently mediated by two unusual nucleotides, guanosine 5'-diphosphate, 3'-diphosphate (ppGpp) and guanosine 5'-triphosphate, 3'-diphosphate (pppGpp)³. These nucleotides are synthesised on the ribosome in the presence of uncharged tRNA, ATP, GTP and an enzyme known as stringent factor³. Mutants which fail to exhibit the stringent response to amino acid starvation with the synthesis of ppGpp pppGpp are known as relaxed mutants⁴ and in *Escherichia coli* are mapped at three loci; *relA* (ref. 5), *relB* (ref. 6), and *rplK* (*relC*) (refs 7 and 8). The synthesis of ppGpp and pppGpp has also been observed in various prokaryotes including blue-green algae⁹. Here we demonstrate the ability of bacterial stringent factor to be stimulated by eukaryotic ribosomal preparations to synthesise both ppGpp and pppGpp.

In eukaryotic cells, viral transformation results in the loss of regulation of many macromolecular processes involved in the response of resting cells to serum deprivation¹⁰. This loss in regulation is reminiscent of that observed in relaxed bacteria. Hershko *et al.*¹⁰ suggested that normal fibroblasts and virally transformed fibroblasts were analogous to stringent and relaxed bacteria respectively. Attempts to find ppGpp and pppGpp in eukaryotic cells have been generally unsuccessful^{11–13}, however, although in the last 2 yr a number of reports describing the accumulation of these compounds have been published, both *in vivo*^{14,15} and in ribosome dependent reactions *in vitro*^{16,17}.

Our inability to duplicate the synthesis of ppGpp in CHO cells as reported by Rhaese¹⁵ and failure to detect ppGpp and pppGpp in a temperature sensitive (*ts*) leucyl-tRNA synthetase mutant of CHO cells at either permissive or non-permissive temperatures¹³, led us to examine the *in vitro* ribosome-dependent reaction reported by Irr *et al.*¹⁷. In this reaction there might be less chance of a rapid degradation of any ppGpp or pppGpp synthesised. Preliminary attempts to duplicate the work of Irr *et al.*¹⁷ using 11- and 14-d-old mouse embryo ribosomes were unsuccessful (Table 1), using either the ribosome assay described by Irr *et al.*¹⁷ or the assay described by Haseltine and Block¹⁸. Addition of purified stringent factor¹⁹ from *E. coli*, how-

ever, resulted in the synthesis of ppGpp and pppGpp (identified by the co-migration with authentic bacterial ppGpp and pppGpp) converting up to 80% of the GTP over a 2-h incubation period into these guanosine nucleotides (Fig. 1, Table 1). The synthetic reaction observed was slightly different from that seen for *E. coli* ribosomes in that the preparations of eukaryotic ribosomes were rapidly able to convert GTP to GDP, which is used in the synthesis of ppGpp (Fig. 1). Synthesis of ppGpp and pppGpp was dependent on ribosomes, GTP, ATP and stringent factor, omission of any one produces less than 0.1% conversion of the added GTP (Fig. 1). Ribosome preparations were not purposely depleted of bound tRNA and mRNA¹⁸, because we desired to keep the mammalian system as intact as

Table 1 Ability of eukaryote ribosomes to synthesise guanosine penta- and tetraphosphate in an *in vitro* ribosome assay

Ribosome source	µg RNA per assay	Reaction time (h)	% of GTP converted to ppGpp and pppGpp	
			Stringent factor	
			40 µg ml ⁻¹	0 µg ml ⁻¹
<i>E. coli</i> salt washed	9.3	2	92.7	<0.1
11-d mouse embryo	1	2	1.1	<0.1
	2	2	44.0	<0.1
	3	3	61.0	<0.1
14-d mouse embryo	1	2	81.3	<0.1
	2	2	18.0	<0.1

Assays were performed as described in Fig. 1. Ribosomes were prepared by the method of Irr *et al.*¹⁷, except the ethanol precipitation step was omitted in all preparations other than the first 11-d mouse embryo experiment. Ribosomal integrity was confirmed by linear (10–40% w/w) sucrose gradient sedimentation analysis²⁰.

possible. Therefore, dependency on these factors was not tested although they were routinely added to the assay.

Lack of a putative stringent factor activity in eukaryotic ribosomes could be accounted for by its lability during the purification procedure for ribosomes described by Irr *et al.*¹⁷. Therefore, ribosomes with various degrees of purity and prepared in other manners were also tested in the *in vitro* assay. We also tested ribosomes prepared from several other eukaryotes. In no case was a stringent factor independent reaction observed, although ribosomes from all sources tested could be stimulated to synthesise ppGpp and pppGpp upon the addition of stringent factor from *E. coli* (Tables 1 and 2). This test also included ribosomes from a *ts* mutant of leucyl-tRNA synthetase in CHO cells²⁰ (Table 1) supporting the *in vivo* data indicating the lack of synthesis of ppGpp and pppGpp in this cell line¹³. The extent of reaction was rather variable (Tables 1 and 2) because ribosomes were not prepared to maintain maximal function but rather by a variety of methodologies in the hope of achieving the independent synthesis of ppGpp and pppGpp by eukaryotes. Therefore quantitative comparisons between groups cannot be made.

Mitochondria from rat liver have been reported to synthesise ppGpp and pppGpp upon the addition of bacterial stringent factor¹⁶. In the present experiments, however, we believe that mitochondrial ribosomes could not be responsible for more than a small percentage of the activity for the following reasons; (1) ribosomes were routinely prepared under conditions where all mitochondria should have been removed, (2) ribosomal preparations showed no 70S peak (<1%) on sucrose gradients even when large quantities of ribosomes were layered on to gradients for preparative purposes.

It is quite possible that the reaction we observed is relatively nonspecific. Crude preparations of *E. coli* stringent factor in the absence of ribosomes can be stimulated to synthesise ppGpp and pppGpp by 20% methanol²¹, although not under the assay conditions employed here (J. Parker, unpublished). Christiansen and Nierhaus²² have shown stimulation of ppGpp and pppGpp synthesis by stringent factor with a variety of *E. coli* ribosomal proteins.

It may be pertinent to point out that immunological cross-reactivity occurs between chicken liver and *E. coli* ribosomal proteins L7 and L12 (ref. 23).

Richter¹² reported that yeast, reticulocyte and calf brain ribosomes were not stimulated by *E. coli* stringent factor. Beres and Lucas-Lenard²⁴ could not stimulate Ehrlich ascites cell ribosomes, but could stimulate wheat germ ribosome with *E. coli* stringent factor. But, as we show here, ribosomes from various sources were stimulated by *E. coli* stringent factor although some of the yeast preparations were inactive. Although the reasons for these discrepancies are not clear, the stringent factor routinely used in the present report was greater than 50% pure whereas in both other reports relatively crude preparations were used. In our hands even a crude preparation of stringent factor could stimulate eukaryotic ribosomes to

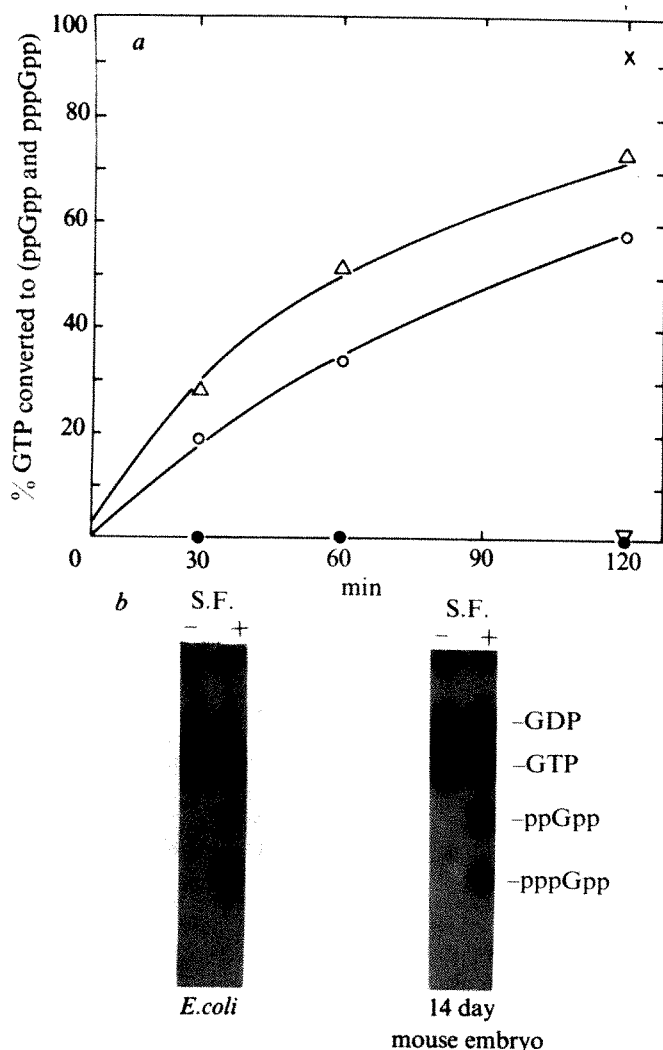


Fig. 1 *In vitro* synthesis of ppGpp and pppGpp by 14-d-old mouse embryo ribosomes and *E. coli* ribosomes in the presence of bacterial stringent factor. *a*, Time course of synthesis of ppGpp and pppGpp. Assays were performed as those of Haseltine and Block¹⁸. 14-d mouse embryo ribosomes were present at a final concentration of 10.4 μg per 50 μl (○), 24 μg per 50 μl (Δ) and *E. coli* ribosomes at a final concentration of 9.3 μg per 50 μl (x). Two μg per 50 μl of at least 50% pure *E. coli* stringent factor (Pedersen and Parker, unpublished) was added where indicated and the reaction mixtures incubated at 37 °C for varying times. The assay lacking ribosomes (●) or lacking *E. coli* stringent factor (▽) gave no detectable synthesis of ppGpp or pppGpp (<0.1% of α -³²P-GTP (Amersham Radiochemicals, specific activity >1 μCi μmol^{-1}) present at 27.5 nmol per 50 μl assay). *b*, A characteristic polyethylene-imine cellulose chromatogram¹⁸ (Brinkman Instruments) of the reaction products of the assay described in (*a*). *E. coli* ribosomes were present at a final concentration of 9.3 μg per 50 μl and 14-d mouse embryo ribosomes at a final concentration of 26.0 μg per 50 μl . Reaction mixtures were incubated at 37 °C for 2 h.

Table 2 Ability of cytoplasmic ribosomes from various sources to synthesise guanosine penta- and tetraphosphate in an *in vitro* ribosome assay

Ribosomes	Synthesis of ppGpp and pppGpp	
	Exogenous stringent factor 40 $\mu\text{g ml}^{-1}$	0 $\mu\text{g ml}^{-1}$
<i>E. coli</i> crude	+	+
<i>E. coli</i> salt washed	+	—
Hamster embryo fibroblasts*	+	—
Polyoma transformed hamster embryo fibroblasts*	+	—
CHO wild type cells†	+	—
<i>ts</i> leucyl-tRNA synthetase mutation of CHO cells permissive temperature 34 °C	+	—
non-permissive temperature 38.5 °C‡	+	—
Tetrahymena	+	—
Yeast	+	—
Rat liver§	+	—

Assays were performed as described in Fig. 1. Stringent factor was present at 40 $\mu\text{g ml}^{-1}$, except with exogenous *E. coli* ribosomes, where no stringent factor was added. Omission of stringent factor gave no detectable spots of ppGpp or pppGpp or autoradiograms and no counts above background (—). The sensitivity of the assay would have detected 27.5 pmol of ppGpp or pppGpp. Positive results (+) ranged from three times background (rat liver) to three hundred and fifty times background (yeast). Ribosomes from tetrahymena, yeast and rat liver were kindly provided by Mr Peter Ganz, Dr W. Galen McKeever and Dr Doris M. Nicholls, York University, Toronto, respectively.

*Ribosomes were prepared by the method of Irr *et al.*¹⁷

†Cells homogenised as described by Stanners and Thompson¹³ and nuclei removed by centrifugation twice at 650g for 3.5 min and mitochondria once at 7,000g for 10 min. A microsomal fraction was obtained by centrifugation for 1.5 h at 105,000g. Pellets were resuspended in buffer C (0.05 M Tris-acetate pH 8.0 at 10 °C, 0.015 M magnesium acetate, 0.06 M potassium acetate, 0.27 M ammonium acetate, 0.001 M EDTA, 0.0001 M dithiothreitol) and frozen at –70 °C until used.

‡Cell supernatants prepared as described by Stanners and Becker²⁹ were layered over 34 ml linear sucrose gradients (15–30% w/w) in 0.01 M Tris-HCl pH 7.4 at 20 °C, 0.1 M NaCl, 0.02 M MgCl₂ and centrifuged for 2.5 h at 27,000 r.p.m. in a SW27 rotor at 4 °C. The monosome and polysome fractions were collected in a LKB fraction collector after monitoring absorbance at 260 nm. Purified ribosomes were concentrated by centrifugation for 14 h in a SW41 rotor at 41,000 r.p.m., resuspended in buffer C and frozen at –70 °C until used.

§The rat liver ribosome preparation converted 91% of the added α -³²P-GTP c.p.m. to inorganic ³²P-phosphate during the course of the reaction.

synthesise ppGpp and pppGpp (unpublished observations).

In conclusion, we find eukaryotic ribosomes are unable to synthesise guanosine penta- and tetraphosphate *in vitro* except in the presence of bacterial stringent factor. Although several methods of ribosome preparation were used to attempt to maintain the activity of a postulated eukaryotic stringent factor no stringent factor activity was found. We cannot, however, rule out the possibility that such a factor exists but is extremely labile. The majority of the *in vivo* experiments^{11–13, 25–28} and our unpublished results argue against this possibility. In light of the present experiments, previous positive results in *in vivo* eukaryotic experiments may well have resulted from prokaryotic contamination of animal cells.

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Quenching of chlorophyll fluorescence by β -carotene

CAROTENOID pigments are closely associated with chlorophyll molecules in the chloroplast structure^{1,2}. Some carotenoids, for example fucoxanthol³, can act as efficient sensitizers for the formation of the excited singlet state of chlorophyll although others, such as β -carotene, are very inefficient^{2,4,5}. Other functions of carotenoids include acting as a deactivator of triplet chlorophyll (and hence protect the chlorophyll from photodegradation and also intercept the production of singlet oxygen) and as a deactivator of singlet oxygen⁶. Here we report *in vitro* experiments which unequivocally demonstrate that β -carotene quenches the excited singlet state of chlorophyll.

A previous report of the quenching of chlorophyll fluorescence by β -carotene⁷ has been shown to be in error^{8,9}. From a simple consideration of the Stern–Volmer quenching kinetics it can be shown that to measurably quench the chlorophyll *a* excited singlet state of lifetime in benzene 5.73 ns, a quencher concentration of $>10^{-3}$ M is required and thus a report¹⁰ of observed quenching of chlorophyll fluorescence by β -carotene at a concentration of 1×10^{-5} M also seems to be in error.

Steady state, quantitative measurements of the quenching of chlorophyll fluorescence by β -carotene are complicated by the fact that the absorption spectra of the two compounds overlap at wavelengths appropriate for excitation of the chlorophyll and also there is overlap of the β -carotene absorption band with the chlorophyll fluorescence. It is therefore necessary to make extensive corrections to the observed fluorescence intensities. The reported results are based on measurements made with 1 cm² cells and cylindrical cells with an internal diameter of 0.2 cm.

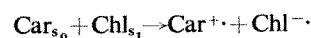
Chlorophyll *a* was used as soon as possible after extraction from *Poa annua* and its purity checked by comparing the relative intensities of its absorption bands at 410, 430, and 662 nm with those in the literature¹¹. For some measurements, chlorophyll *a* from Sigma Chemicals was used: spectral properties of the extracted and commercial samples were identical. Synthetic all *trans* β -carotene (Sigma) was used and its purity checked by measuring the extinction coefficient of its absorption band at 450 nm. The shape of the absorption curve matched, in details, with the literature spectrum for the compound¹¹. The absorption

spectrum of the β -carotene used in the experiments was independent of the concentration used. For all the concentrations used there was no apparent deviation from Beer's law. The linearity of the Stern–Volmer is a further indication that β -carotene does not aggregate at the concentrations employed. Spectrograde benzene (BDH and Merck Uvasol) was used as solvent. For measurements in the square cells, the chlorophyll solution had an optical density of 0.39 at the excitation wavelength (662 nm). The fluorescence intensity was measured at 670 nm. In all cases the solutions were air-saturated. The results of using the square and cylindrical cells are shown in Fig. 1. The agreement between the two sets of results is not very good but the values show that the quenching process has a rate constant of $\sim 5 \times 10^9$ M⁻¹ s⁻¹ which is close to the diffusion controlled limit of 1×10^{10} M⁻¹ s⁻¹ (ref. 13).

To check the steady state quenching data, measurements were made of the quenching effect of β -carotene on the lifetime of the excited singlet state of chlorophyll *a*. Fluorescence lifetimes were measured using time correlated single photon counting¹⁴. The solutions were excited at 550 nm and the emission at 670 nm monitored with a Phillips 56 TUVP photomultiplier tube. The fluorescence was observed at the back of a 1 mm path length cell. In all cases the fluorescence decays were exponential. The results are shown in Fig. 1. The rate constant for quenching, 4.5×10^9 M⁻¹ agrees well with the steady state measurements.

A surprising observation was that the admixing of β -carotene and chlorophyll *a* in aerated solution gave rise to chemiluminescence. The emitting species (as shown by its spectrum) is the excited singlet state of chlorophyll. This luminescence may arise as a result of the thermal dissociation of a β -carotene oxidation product (perhaps a 1,2-dioxetane) to give products in their excited singlet state. Förster-type energy transfer from these excited molecules to β -carotene should occur and ultimately some of the energy be trapped by the chlorophyll lower excited singlet state from which fluorescence occurs.

Since the energy of the excited singlet state of chlorophyll lies below that of β -carotene, the quenching of chlorophyll fluorescence cannot be attributed to an energy transfer process. From the fact that polyenes such as β -carotene have low ionisation potentials and are known to act as electron donors towards excited singlet states¹⁵, we propose that the quenching occurs via an electron transfer process,



The initially produced intermediate may be a non-emissive exciplex. If the quenching occurs in a polar environment radical ions should be produced. Whether or not these can be used in the photosynthetic process is dependent on a number of factors. Of particular importance is the likelihood of back electron transfer before the ions have diffused out of the solvent cage in which they were generated. This type of reaction readily occurs when the radical ions are generated from excited singlet states^{16,17}. We have proposed a similar mechanism to account for the quenching of methylene blue fluorescence by β -carotene¹⁸. Since there is only a little evidence¹⁹ for chlorophyll *a* acting as an electron acceptor in its singlet state we studied the quenching of its fluorescence by other donors, *N,N*-dimethylaniline, indole and skatole. The quenching rate constants for the compounds in methanol solution were found to be 3.4, 0.3 and 0.3×10^8 M⁻¹ s⁻¹ respectively and for indole in benzene solution a value of 3×10^8 M⁻¹ s⁻¹ was obtained.

At first sight, the reported result is in direct conflict with the many well established results on the *in vitro*²⁰ and *in vivo*^{21,22} sensitisation of chlorophyll fluorescence by carotenoids. Because many carotenoids exhibit only very weak fluorescence in solution, energy transfer was demonstrated by an indirect method—the excitation spectrum of the fluorescence of chlorophyll *a* in a chlorophyll– β -carotene mixture was shown to contain bands due to β -carotene²⁴. These experiments cannot,

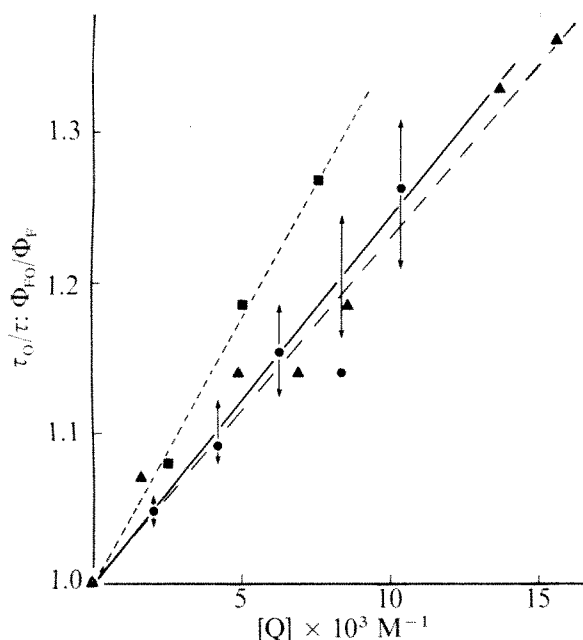
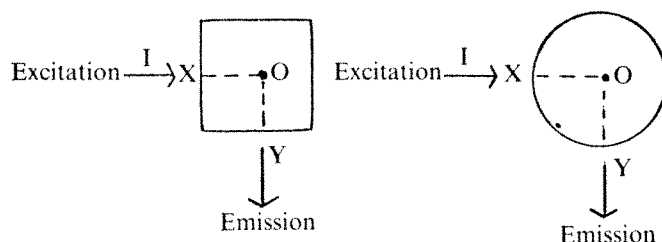


Fig. 1 Quenching of the fluorescence, and fluorescence lifetimes of chlorophyll *a* by β -carotene. Δ , τ_0/τ (— best fit K_{SV} 22.5); $\bullet$, Φ_{F0}/Φ_F (square cells, — best fit K_{SV} 26 $\pm$ 5); $\blacksquare$, Φ_{F0}/Φ_F (cylindrical cuvette — — — best fit K_{SV} 37 $\pm$ 5). τ and τ_0 , Chlorophyll *a* lifetime in presence and absence of β -carotene. Φ_F and Φ_{F0} , Chlorophyll *a* fluorescence quantum yields in presence and absence of β -carotene. $[Q]$, Quencher concentration; K_{SV} , Stern-Volmer quenching constant. Φ_{F0}/Φ_F was calculated by the following procedure: consider the fluorescence from the centre O of a cell, illuminated at X by light of intensity I , and observed at right angles at Y.



In the absence of a quencher let the transmittance of the solution over XO and OY be x and y respectively. Then the fluorescence intensity at Y, $F_0 = Ixy\Phi_{F0}$. If the transmittance of a solution of pure quencher over XO and OY is α and β respectively, then the new observed fluorescence intensity of quenched chlorophyll solution, $F_{obs} = Ixy\alpha\beta\Phi_F$. Hence the observed Stern-Volmer quenching $F_0/F_{obs} = \Phi_{F0}/\alpha\beta\Phi_F$. $\alpha = 10^{-\epsilon_1 c l}$ and $\beta = 10^{-\epsilon_2 c l}$; ϵ_1, ϵ_2 are extinction coefficients of β -carotene at excitation and monitored emission wavelength respectively; c is quencher concentration; l is distance from centre of cuvette to X and Y (normally equal).

because of their design, show whether the quenching process occurs.

We propose that the role of carotenoids in photosynthesis is wider than originally envisaged. On interaction of the excited singlet state of β -carotene with chlorophyll the energy can be partitioned between energy transfer and an electron transfer process. The relative importance of the two processes will be determined by the orientation and the distance separating the two molecules. Since energy transfer occurs by a resonance process²⁰⁻²², it should be favoured relative to the redox process by an increase in the distance separating the two molecules. The environment of carotenoids in the antennae is known to be different from that in the reaction centre^{24,25}. In the antennae resonance transfer from carotene to chlorophyll and rapid resonance transfer from one chlorophyll molecule to another may compete successfully against the electron transfer reaction such that only resonance transfer is occurring. At the reaction

centre, resonance energy transfer from the centre to a chlorophyll molecule outside the centre becomes inefficient because of the lower energy of the excited singlet state of the chlorophyll in the centres. Consequently, the electron transfer should be favoured and a chlorophyll radical anion and a carotene radical cation formed. This latter species will undergo a rapid ground state reaction with a neighbouring chlorophyll molecule^{22,26} to generate a chlorophyll radical cation²⁷ and thus the overall effect of the electron transfer reaction is to produce a charge separated pair Chl^-Chl^+ . It has been proposed that such a dimer species is involved in photosystem II (ref. 25).

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Corrigendum

In the article 'Economics of alternative energy sources' by Martin Ryle, *Nature* **267**, 111-117, the figures given in Table 1 under Present Gas Turbine Generators should have included two entries: *a*, the contribution from oil-fired steam stations, showing an annual variation and *b*, the contribution from gas turbines used for peak boosting. These figures were inadvertently combined and used with the gas turbine time variations. The correct figures lead to a reduction in the final peak value in the Table from 187 to 163 GW and a reduction in the installed capacity needed from 266 to 233 GW; these figures are still some 50 times those of the present nuclear capacity.

matters arising

Culture and genetic variability

CHAKRABORTY found¹, by using gene frequencies for seven serological markers to compute genetic distance, that 'only geographical distance seems to be important, explaining the trend in genetic variabilities' in seven Chilean Indian populations. The correlation coefficients he presented were 0.716, 0.775, and 0.901, for correlations between genetic distance-geographical distance, genetic distance-cultural dissimilarity, and geographical distance-cultural dissimilarity, respectively. He then presented the results from a stepwise regression, and found that while geographical distance explained 51.31% of the genetic variation, cultural dissimilarity explained only an additional 8.88%. He did not, however, use as the first variable in the stepwise regression the one which had the highest correlation with the dependent variable. Culture alone accounts for 60.06% (r^2) of the genetic variation in these data. If geographical distance is then added, the additional variation explained would be less than 0.2%. These figures are, of course, approximations subject to slight rounding error from the published tables. It is indeed difficult to assess the relative importance of variables which are themselves highly correlated, as Chakraborty states. But his data clearly do not support his conclusion that of the variables measured, only geographical distance is an important correlate of genetic distance. From these data, culture is at least of equal importance.

It would be profitable to use these data to consider the relative effects of genetic and geographical distance upon culture. Geographical distance apparently explains 81.18% of cultural diversity as measured in this study. This leaves a smaller, but possibly significant, proportion of cultural variation which might be explained by the genetic measure.

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CHAKRABORTY REPLIES—Greene¹ contests my assertion regarding the relative importance of geographical versus cultural separation as a correlate of genetic variation in seven Chilean populations². In so doing, she notes that in the stepwise regression procedure I used geographic distance as the first variable even when the cultural dissimilarity index had a higher product moment correlation with genetic distance (the dependent variable). Two reasons justify my choice of geography as the first independent variable to be entered in the regression analysis, one being theoretical, the other pragmatic.

Take, for instance, cultural variability as the dependent variable. Evaluation of the relative effects of geographical and genetic distances on cultural variability indicate that 82.41% of the cultural variability is explained by geographical distance ($P < 0.0005$), whereas the additional effect of genetic distance is 4.01%, which is statistically insignificant ($0.10 > P > 0.05$). This alone may suggest that geographical distance should be the first variable of interest.

The pragmatic view concerns the choice of the characteristics determining cultural diversity. As elaborated elsewhere³, it is difficult to ascertain that cultural attributes are independent of geography. For example, we classified the seven populations on the basis of their types of subsistence, housing and architecture, economics, mode of gathering food, etc., which do have strong geographic components embedded in them. Thus, the strong association between cultural affinity and geographic proximity is indeed historical in these seven populations.

Furthermore, a stepwise regression method often under-represents the importance of the last variable to go in, particularly when the independent variables are significantly correlated with the dependent one. A logical explanation of causal relationship in such an event has to be based on an understanding of the process producing the covariation between the variables, and not based on which independent variable has the highest correlation with the dependent one.

Greene's criticism, therefore, should be viewed with caution, although any generality of my analysis needs further examination, possibly from studies of

other populations with comparable ethnographic accounts.

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² Chakraborty, R. *Nature* 264, 350-352 (1976).

³ Chakraborty, R., Blanco, R., Rothhammer, F. & Llop, E. *Soc. Biol.* 23, 73-82 (1976).

Do viruses use calcium ions to shut off host cell functions?

IN discussing the shut-off of host cell functions by poliovirus, Carrasco and Smith¹ ask: "How does a viral protein change the ionic environment inside the cell?" Perhaps it forms channels across the external membrane². For example, a viral structural protein might generate holes by assembling into hexamers, pentamers, or extended lattices in a membrane.

Although Carrasco and Smith emphasised Na^+ ions, divalent ions are probably more important controllers of metabolism. In general, Ca^{2+} , Mg^{2+} and polyamine ions tend to form much more specific links with polyelectrolytes than Na^+ or K^+ do³, and the Debye-Hückel concept of ionic strength grossly underestimates the relative binding of, say, Ca^{2+} compared with K^+ , to membranes and viruses⁴. Many enzymic activities depend upon divalent ions, while relatively few respond to physiological changes in monovalent ion concentrations.

Of the two main divalent ions in biology, it is Ca^{2+} , not Mg^{2+} , that usually acts as a signal. This is because Ca^{2+} is actively pumped to at least a thousandfold concentration difference across membranes⁵, whereas Mg^{2+} is much nearer equilibrium⁶, probably because it permeates more easily. The fact that cells respond more to changes in extracellular Mg^{2+} than Ca^{2+} has misled some workers into putting a false emphasis upon the ions' relative roles⁷.

The early effects of many lytic viruses can readily be interpreted as a consequence of increased flow of Ca^{2+} into

¹ Chakraborty, R. *Nature* 264, 350-352 (1976).

cells. In some cases structural components of the virus perhaps act as the ionophores^{1,8,9}, while in others new proteins may need to be synthesised. The many phenomena observed in practice would then be merely the different facets of a fundamental gear-shift in polyelectrolyte-counterion interactions within the cell.

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CARRASCO AND SMITH REPLY—We find the proposal by Durham that changes in calcium ion concentration following infection by viruses are responsible for alterations in host cell functions very attractive. Our model to explain the shut-off of protein synthesis, which was briefly summarised in our *Nature* paper¹, and which is the subject of a more detailed paper², is in fact very similar. We also favour a model in which viral structural proteins form lattices in the cellular membrane and leave holes which act in the same way as some ionophores to allow the passage of ions².

We believe, however, that the shut-off of host cell protein synthesis is best explained by a change in Na⁺ ion concentration within infected cells, and we presented experimental evidence showing that sodium ions can have a differential effect on protein synthesis *in vitro* and indirect evidence that such changes in sodium ion concentration could occur *in vivo*¹. While we have not examined the effects of calcium ions in detail, a preliminary screening of the effects of many mono- and divalent cations on protein synthesis *in vivo* and *in vitro* failed to reveal a differential effect by calcium ions.

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Sodium emission in persistent meteor trains

USING theoretical estimates of the rate coefficient of the association of Na with O₂, Kolb and Elgin¹ have shown that the catalytic effect of sodium in releasing the store of recombination energy of free atmospheric atomic oxygen is more powerful than was previously assumed². Drawing on evidence from measured Na D-line intensities, Kolb and Elgin estimate a branching ratio for the production of excited Na(²P) from NaO reduction of $f \sim 0.05$, which, using the model previously suggested² implies that at 90 km sufficient photon emission occurs for an enduring train to result from a meteor of magnitude about –6. It is instructive to relate this estimate to observation. It is known that the trains of small duration show an initial emission decay at 90 km of about³ 0.2 mag s^{–1} and since the radiation probably results from the interaction of ionic constituents of the train so that the reaction rate varies inversely as the meteor column cross-sectional area and hence inversely as time, a decay of 5 mag would be expected between about 1 s and 100 s. We may infer that any train luminosity having a duration $t \gtrsim 100$ s must be due to a catalytic (sodium) mechanism. In Olivier's catalogue⁴ 55% of trains ($t > 10$ s) were produced by high velocity shower meteors, Perseids, Orionids and Leonids. In the duration-magnitude characteristics given for 3700 trains by Millman⁵ the average meteor magnitude corresponding to a duration of 100 s is in the range –7.5 to –5 for velocities 60–70 km s^{–1}. From the extensive surveys of Hoffmeister (see ref. 4) and Olivier, one visual meteor in 780 results in a train of duration $t > 10$ s. Using ref. 5 the difference in magnitudes between meteors responsible for trains of 10 s and 100 s duration is 2.5 implying a corresponding incident meteor flux ratio⁶ of 27:1. Since the mean visual meteor rate⁶ to a single observer is 9.7 h^{–1} the observed occurrence frequency of trains $t > 100$ s is 4.6×10^{-4} h^{–1}. In comparison the cumulative flux of meteors having magnitudes brighter than –6 for an individual observer is, using Hughes⁷ 3.1×10^{-4} h^{–1}. Though there are uncertainties in [O₂] and f and variations in Na abundance, the sodium cycle process is strongly supported as a source of persistent meteor train luminosity. This conclusion may be viewed in the light of the review of train characteristics by Hughes⁷.

Hughes considered evidence for the mechanisms responsible for persistent meteor train emission and on the basis of visual observations^{8,9} concluded that

the evidence supported a mechanism that is closely associated with the level of meteoric ionization rather than with meteoroid mass or meteor luminosity. It is of importance to emphasise that based on the observations^{8,9} the conclusion of Hughes is inappropriate. The results of Lindblad⁸ for Perseid meteor trains are confined to very short durations, t : for only 2% of the trains was $t > 3$ s while for 90% $t < 1.5$ s indicating that the observed light was associated with the meteor wake emission and in particular the well known OI 5577 Å feature. Indeed the data of Lindblad permit the determination of the effective lifetime τ (decay constant) of the emitting species. It is straightforward to show that the gradient of train duration-meteor magnitude plot is given by $-\tau(\log_{10})/1.51$ and using Lindblad (Fig. 11B) $\tau = 0.21 \pm 0.05$ s. In comparison, the radiative lifetime of the OI (¹D₂ ← ¹S₀) transition is 0.74 s. However the great majority of observed trains were in the 90–100 km height interval (Lindblad, Fig. 18) where deactivation of the O(¹S₀) state most effectively occurs with O and O₂. Using recommended quenching coefficients and known atmospheric concentrations the quenching rate at 95 km is ~ 2.9 s^{–1} resulting in an effective emission lifetime of 0.23 s. Plavec⁹ does not state the durations of trains in his analysis. We note, however, that according to Plavec (a), the trains disappeared in a few seconds, (b), there was concern with the effects of observer reaction times on duration measurements (c), 50% of Perseid meteors yielded trains in contrast to the much lower proportion at lower meteor velocities. The results indeed coincide with what is now known about the green line emission (the 5,577 Å feature was not identified until 1958). It is quite clear that the observations of Lindblad and Plavec do not refer to the true rare enduring train phenomenon.

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reviews

Thinking about complexity

Eric Ashby

Tools for Thought. By C. H. Waddington. Pp. 250. (Jonathan Cape: London, 1977.) £5.95.

HAL WADDINGTON did not live to see this book through the press. It is a fitting valedictory from one of the few of my contemporaries who deserve the epithet 'Renaissance Man'. Thirty-six years ago he published a little book called *The Scientific Attitude*, which delighted us by weaving poetry and art (with illustrations from Auden, Spender, Picasso and Paul Klee, among others) into a fabric of closely knit rational argument about science. For a generation he continued to carry out distinguished research in biology, and at the same time to publish thoughtful and stimulating work on ethical and social issues. In February 1975 he presided over an imaginative symposium at the Ciba Foundation, on *The Future as an Academic Discipline*. Over morning coffee he showed me the rough drafts of two books which were to embody his recent thinking. One draft was on *Man-Made Futures* (alas, not sufficiently advanced to be published posthumously); the other draft was *Tools for Thought*.

The title of the book accurately describes its content. Open your newspaper any morning and you'll read items such as these: conflict over transport policy for London; industrial dispute over the introduction of new equipment for typesetting; threat of a strike among sewage workers; apprehension about aerosols and ozone, or saccharine and cancer; alarm about population growth; warnings about depletion of resources; diplomatic hassles in the Middle East. We suffer saturation bombardment with advice about these issues, panaceas, utopian scenarios. What we rarely get is help on how to think about them. Waddington's book offers this help.

All these issues, and scores of others like them, have one property in common: they are embedded in a network of complexity. If doing *a* simply caused *b*, which in turn simply caused *c*, with no side-effects, how simple these issues would be to understand! But the tool

of linear thinking is useless on problems of complexity. It is well known that there are now new tools for thought—labelled systems analysis, information theory, decision theory, cybernetics, and so on. But most people who have not had to use these tools for professional purposes know very little about them and are put off by the turgid jargon in which they are packaged.

The purpose of Waddington's book is to provide a plain man's guide to these new tools for thought. It's not an instruction manual to teach you how to use them, but it demonstrates their capacities and their limitations, and it provides enough concrete examples to convince the plain man that what Waddington calls "Conventional Wisdom of the Dominant Group" (with the felicitous acronym COWDUNG) is often totally inadequate to handle contemporary social and technological problems.

After an introduction which identifies Waddington as a neo-Whiteheadian the book describes the conceptual tools for thinking about complex systems. First, it deals with shapes, with an illuminating digression on hierarchies and the use of matrices and Venn diagrams. It then deals with processes: a lucid exposition of exponentials and their consequences, sigmoid curves, feedback, chain reactions, the unexpected phenomena like schismogenesis discovered by Gregory Bateson, and an inkling of the way topologists describe complex social interactions by the use of catastrophe theory. After processes come communications, with a summary of some of the astonishing consequences of programming patterns into a computer. Communication leads to games theory and operational research, and this in turn to technological forecasting and system modelling (the one section of the book which I'm sure Waddington would have wished to revise if he had had the opportunity).

This is a catalogue of techniques daunting enough to repel the plain man for whom it is written. But my guess is that the plainest of men will find the book hard to put down. One of its delights is that it is so easy to read and

yet Waddington hasn't fudged any of the intellectual difficulties. The style is informal, conversational, as if the book had been taped direct from lectures. It combines a rare gift for popularisation with uncompromising scientific integrity; several times in the book Waddington as good as says: "I've shown you how this tool is used, but don't imagine you now know how to use it yourself. You don't." Just as the reader may be becoming puzzled at the description of some concept, Waddington throws in a specific example which dispels the puzzlement. Thus, in discussing the "prisoner's dilemma" (that theme so dear to philosophers and so ludicrous to the plain man), Waddington suddenly says that this is the paradigm for discussion about urban transport (I'll give up my car if everyone else gives up his car), grazing rights (I'll run fewer cows on the common if the others do the same), and the East-West arms race.

The book is illustrated by diagrams, most of which are deliberately imprecise because, as Waddington writes in the introduction, they are there to illustrate ideas, not facts. I see his point but I'm not happy about these diagrams. They are the sort of crude sketches you'd make on the blackboard while talking to a class. Some of them are insufficiently explained in the text. I may be pernickety, but in an otherwise elegantly printed book, I would like the lines marking the ordinates and abscissae of graphs to be straight. Graphs can be informal, but nevertheless tidy (as they are, for instance, in Hogben's *Mathematics for the Million*).

Hal Waddington was not able to correct the proofs of this book. Whoever did correct them ought to have taken more care. There are several misprints, some of them serious (for example, the date of Waddington's Bernal Lecture on p237). The diagram on p99 is upside down. I believe a diagram is missing altogether on p202, and possibly also on p220 or p221. The figure on p214 is inconsistent with the text. An *n* is missing from the equation on p207. Many of the diagrams could

be improved not only by a judicious use of a ruler to draw straight lines but by the addition of legends to supplement references in the text.

The book is, in my view, a brilliant essay for the plain man, particularly for the plain politician and administrator, who wants to understand how experts study complexity. It is a generous bequest from one of the most distinguished biologists of his generation. It deserves to be re-issued for a

wider public as a paperback at a moderate price; but it would be a courtesy to the memory of Hal Waddington to re-edit the book conscientiously before it is re-issued. □

Lord Ashby was formerly Master of Clare College, Cambridge. He is Chancellor of the Queen's University, Belfast, and is at present Walgreen Professor of Human Understanding at the University of Michigan.

Mathematical treasure

Proofs and Refutations: The Logic of Mathematical Discovery. By Imre Lakatos. Edited by John Worrall and Elie Zahar. Pp. xii+174. (Cambridge University: Cambridge, London and New York, 1976.) Hardcover £7.50; paperback £1.95.

For anyone interested in mathematics who has not encountered the work of the late Imre Lakatos before, this book is a treasure; and those who know well the famous dialogue, first published in 1963-64 in the *British Journal for the Philosophy of Science*, that forms the greater part of the book, will be eager to read the supplementary material. The dialogue is what Lakatos calls a "rational reconstruction of history", that is, the history of the Euler conjecture on polyhedra ($V-E+F=2$); the purpose is to illustrate principles of methodology. The participants seem themselves to be more interested in methodological questions than in the mathematical problem, which imparts a somewhat ponderous tone to the discussion; but the reconstruction, and the actual historical information given in the extensive footnotes, are in themselves fascinating. The supplementary matter, extracted by the editors of this posthumous work from earlier unpublished writings by Lakatos, and not revised by him before his death, consists, first, of a conclusion to the dialogue, in the form of a discussion of Poincaré's proof, and, secondly, of much briefer case studies, not in dialogue form, of examples from analysis and measure theory.

The original dialogue, without this supplementary material, was stimulating but tantalising, in that it was unintended his readers to draw. Plainly, clear just what conclusions Lakatos one is a methodological thesis, concerning the process of mathematical discovery. According to Lakatos, the best model for this process is not the linear, Euclidean one of indubitable deductions from an initial set of axioms and

definitions, but that of an interplay between ideas for proofs and counter-examples in the light of which the proof is progressively improved. We begin, typically, with a conjecture and a tentative proof. We should then seek for counter-examples, guided by the proof: when these are found, we should not rule them out by *ad hoc* ("monster-barring") definitions, even when it can plausibly be claimed that our original concepts, as we vaguely apprehended them, did not cover those examples; nor should we merely make an ("exception-barring") restriction of the domain of application of the theorem so as to exclude the counter-examples. Rather, the proof must be re-analysed in the light of the counter-examples: in this way, we discover hidden lemmas, and build into the statement of the theorem the hypotheses essential for its validity.

With these maxims about how to do mathematics, few would disagree, despite a certain truculence in Lakatos's manner of arguing for them. He has, however, another thesis, about how mathematics should be presented. He deplores the deductivist Euclidean style, in which a set of complicated definitions and axioms are stated at the outset, without explanation, and complex theorems follow, with their proofs in the barest form. Exposition in this manner conceals what is required for understanding: instead, it should reflect the actual history of the problems, "rationally reconstructed", so that the motivation of the definitions, axioms and hypotheses of the theorems becomes apparent. This second thesis, about mathematical style, is more tendentious than the first, but has a great deal to be said in its favour. Certainly it impedes understanding when a definition is given without any illustration of the cases to which the term so defined does and does not apply, and without any explanation why it should be framed as it is; and certainly also the historical information embedded in most mathematical textbooks is often mythological. How much difference would be made to the best mathematical expositions by the adoption of

Lakatos's recommendations depends on how much is allowable as "rational reconstruction".

The most important question, however, is how far Lakatos intended to assert, as well as a methodological and a stylistic thesis, a philosophical one. Most people's first reaction to Lakatos's work is to say that the situation he describes is one likely to prevail in a branch of mathematics in its early stages, before it has attained the condition of an axiomatised theory, but not when it has reached maturity. On this view, Lakatos's observations have no philosophical consequences: they describe how we do, or can best, arrive at a developed theory, how we can make mathematical discoveries, not what such a theory is like when we have succeeded in framing it. There are, however, passages (pages 100 and 138) suggesting that Lakatos took a quite different view, namely that the process of finding counter-examples and re-analysing proofs in the light of them is never-ending, or, at least, that we can never know that we have reached an end.

This thesis, if accepted, would indeed be of critical philosophical importance, since it would demand, though it would not itself provide, a revision of most of the known accounts of mathematical knowledge, if not of mathematical truth. Other passages (for example, p. 124), however, suggest that the process does terminate. The editors are more clearly of this opinion than Lakatos himself (pages 125-6 and 138n). They think that, by formalisation, one can dispel all doubt from the proof, and throw it upon the axioms: from these, however, it can never be removed. This leaves a big lacuna in the whole discussion: the character of doubt concerning the axioms of a mathematical theory is far from obvious. Thus, in the upshot, we are presented, in this book, with a piece of work which, although admirable as a historical study, is, from a philosophical standpoint, incomplete and inconclusive. Doubtless the delay in publication was due to Lakatos's awareness of this: had he lived, we should presumably have eventually had a more finished work.

Nevertheless, the book, as it stands, is rich and stimulating, and, unlike most writings on the philosophy of mathematics, succeeds in making excellent use of detailed observations about mathematics as it is actually practised.

Michael Dummett

Michael Dummett was Reader in the Philosophy of Mathematics at the University of Oxford from 1961-74, and has been Senior Research Fellow at All Souls' College, Oxford, UK, from 1974.

Ramifying phylum

The Biology of the Mollusca. By R. D. Purchon. (Second edition.) Pp xxv+560. (Pergamon: Oxford and New York, 1977.) £17.50.

ARISING from a common stock with the segmented annelids and arthropods, the molluscs proceeded to evolve along totally different lines, to attain in the cephalopods the culmination of invertebrate organisation. They did so from a basis of possibly secondary simplicity—this depending on whether or not the enigmatic *Neopilina* may be regarded as segmented.

Contemplation of the structure of early fishes might have led some contemporary evolutionist to predict developments in structural pattern that could lead to amphibious and eventually terrestrial success. Similar survey of the first obscurely crawling molluscs could have afforded no faintest indication of possibilities eventually realised in the torsionally produced gastropods, compressed and insinuating bivalves, and predacious, rapidly darting squids.

The consequences of the radiating evolutionary possibilities of primitive molluscan structure are admirably revealed in this second edition of a book which received all too little notice when first published in 1968.

Although a sizeable volume it cannot hope to cover the enormous extent of form, function and environmental

specialisation achieved by members of this intricately ramifying phylum, with convergence running wild to produce both snail-like bivalves and bivalve gastropods. The author has concentrated on groups or aspects with which he is most at home or which he feels are of greatest significance.

Almost complete adherence to the original pagination has prevented any extensive introduction of new material. Due note has, however, been taken of the recent work of Professor J. A. Allen on the Verticordiidae. These latter bring the septibranch bivalves into unquestioned association with the eulamellibranchs and exclude previous ideas about possible direct connection with the protobranchs.

The precise nature and course of the digestive processes in the bivalves continues to provoke investigation. Here, Professor Purchon provides a judicious review of the present state of the controversy, largely stimulated by the work of Dr Brian Morton, on the precise cyclic nature of these processes. He has also brought up to date his sections on the distribution, and mode of spreading, of gastropods on Pacific islands.

This new edition provides an admirable introduction to the advanced study of this significant phylum.

C. M. Yonge

Sir Maurice Yonge is Honorary Fellow in Zoology at the University of Edinburgh, UK.

Fusion research

Nuclear Fusion. Special Supplement, 1976. (World Survey of Major Facilities in Controlled Fusion Research.) Pp. 866. (International Atomic Energy Agency, Vienna, 1976.) \$48.

THIS large volume provides a unique insight into the magnitude and variety of the world's research and development effort in nuclear fusion. Following the false dawn at the time of the early Geneva Conferences, an immense amount of experimental and theoretical work by the plasma physicists has led to the construction of numerous plasma-confining devices, quite apart from more recent initiatives on the entirely distinct concept of laser-implosion devices. Now several laboratories, notably Argonne and Oak Ridge in the United States have embarked on long-range (20 years plus) development programmes of operating fusion reactors on the basis of the tokamak confinement principle, incorporating detailed development programmes in all the subsidiary technologies from ultrahigh power electrical supplies to tritium processing.

The World Survey first classifies programmes by country and laboratory; each entry specifies purpose, key personnel, main features, major results and future plans. Numerous perspective drawings of actual or conceptual reactor designs are included, the Americans, Russians, Japanese and Germans seeming to have the largest and most varied programmes. There follows a summary section classified by types of programme (confinement systems, plasma physics, heating techniques and fuel injection strategies, reactor considerations including materials research, and laser research—including laser-aided fuel injection into plasmas). There is an alphabetical index of more than 3,000 key scientific workers.

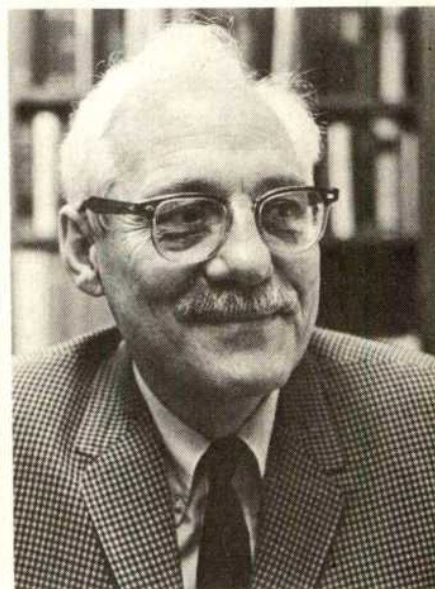
It is a major achievement to have assembled so much solid information in standardised format from so many sources. The scale and scope of the work described implies the growing optimism that nuclear fusion represents a genuine hope as an energy source for the twenty-first century.

Robert W. Cahn

Robert W. Cahn is Professor of Materials Science at the University of Sussex, UK.

The May issue of Nucleic Acids Research

is devoted to papers written by former colleagues and friends of Jerome Vinograd (1913–1976). This memorial issue is respectfully dedicated to him.



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Population analysis

Spatial Population Analysis. By P. H. Rees and A. G. Wilson. Pp. 356. (Arnold: London; Academic: New York, 1977.) £27.50; \$35.50.

ALL would agree with the authors' aim to provide good methods of population analysis for effective social and economic planning; this detailed and thorough text provides such an essential basis. In their preface, the authors warn the reader that he must face considerable conceptual difficulties in acquiring the skill, but comfort him by claiming relatively little demand on formal mathematical knowledge apart from algebra and an elementary knowledge of matrices. Nevertheless, this is a formidable text that needs to be painstakingly worked through to follow the argument.

The strength of the text is claimed to be that for the first time the analysis takes full cognisance of migration through the "use of new and powerful conceptual and mathematical tools". Such recognition of the importance of migration is welcome, since it has received scant treatment in some recently published work, a strange weakness in a world of growing mobility.

The text itself is arranged in five parts, each well introduced, and the argument subsequently explained and documented. The first part introduces current population models and their associated problems: here the authors introduce one of their fundamental features—models based on age groups and not life-tables, a basic divergence from the work of Keyfitz on the mathematics of population. It is also pointed out that although concentration is on theoretical concepts, examples using real data are introduced to illustrate the argument.

The second part introduces the concept of "population accounting" and is illustrated by reference to aggregate populations. The third part is devoted to the development of realistic population models, followed in the fourth part by the putting forward of the concept of life-table accounts to demonstrate that spatial dimensions may be added to conventional life-table analysis to make associated models more systematic, consistent and rigorous. The final, fifth, part summarises the new methods and their applications as well as their relationship to development in other fields.

The authors have been careful to make sure their work can be applied empirically by limiting themselves to commonly available data, although

they have interwoven their own work with that of Lexis, Pressat, Rogers, Stone and others already accepted among demographers.

With page after page of mathematical formulae and matrices, the publishers have faced a monumental task in producing the book in present conditions at a realistic price. It is no wonder that the book is nevertheless expensive.

R. E. H. Mellor

R. E. H. Mellor is Professor of Geography at the University of Aberdeen, UK.

γ -ray astronomy

Gamma-Ray Astronomy. By E. L. Chupp. (Reidel: Dordrecht, The Netherlands and Boston, Massachusetts, 1976.) Cloth Dfl 105; paper Dfl 50.

THE title on the cover of this book reads *Gamma-Ray Astronomy* but inside a subtitle, *Nuclear Transition Region* has been added. This uncertainty as to the scope of the book extends into the text and gives an uneven quality to what is, otherwise, a valuable contribution to this field of astronomy.

The first chapter is a brief introduction and it is followed by a chapter on the fundamental mechanisms which produce γ rays. Those processes which lead to line spectra, such as transitions between excited states of nuclei and the annihilation of positrons, are treated very thoroughly. Presumably for the sake of completeness this chapter also deals with mechanisms such as Compton scattering, *bremstrahlung* and the decay of neutral pions, all of which lead to the production of continuous spectra.

The mechanisms discussed in chapter 2 are then used in the next chapter to predict the γ -ray emission which may be expected from astronomical sources. The inclusion of a chapter on predicted fluxes was perhaps necessary because of the paucity of experimental data in γ -ray astronomy, but this does lead to a certain amount of repetition in the book because some of the discussions are presented again when the experimental results are described in chapter 5. The author's interest in continuous spectra seems to waver a little in chapter 3 and he provides only a brief discussion of the high energy γ -ray emission from pulsars and supernova remnants, and makes no mention of extragalactic sources such as radio galaxies and quasars. Chapter 4 is concerned with the interactions of γ rays with matter and is included as an introduction to

experimental problems in γ -ray astronomy.

Chapter 5 is devoted to observational results. As in chapter 3, the treatment of the sources of continuous spectra is not consistent; for example, no mention is made of the many measurements of the pulsar NPO532 in the Crab nebula although the unsuccessful searches for γ rays from extragalactic supernovae are described.

Chapter 6 deals with experimental problems in γ -ray astronomy and, appropriately, is the longest chapter in the book. It contains a comprehensive discussion of the background problems in a wide variety of detectors; if the quantity of data referred to does seem a little forbidding to the newcomer to the field, it will at least remind him of the need for technical innovation if this branch of astronomy is to make significant progress.

This is the first book to place the correct emphasis on the experimental problems in γ -ray astronomy and will therefore be invaluable both to the newcomer and to the experienced worker in this field.

R. R. Hillier

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announcements

On the move

S. K. Banerjee, Professor of Geology and Geophysics at the University of Minnesota, is on sabbatical leave from 1 September 1977 to 30 June 1978, as a Visiting Scholar in the Office for History of Science and Technology, University of California, Berkeley, and a Visiting Professor in Geophysics at both Stanford University and U.C. Berkeley. Communications to 722 Wildcat Canyon Road, Berkeley, CA 94708.

A. Albert, Research Professor at the State University of New York at Stony Brook, to Emeritus Professor in the Research School of Chemistry, Australian National University, Canberra, P.O. Box 4, A.C.T. 2600, Australia.

Appointment

Professor J. G. Beetlestone, to the recently created Chair of Science Education in the Department of Education at University College, Cardiff.

Members of the National Academy of Sciences elected on 26 April 1977

G. A. Almond, Stanford University. **M. Artin**, Massachusetts Institute of Technology. **F. E. Bloom**, The Salk Institute for Biological Studies, San Diego, California. **W. S. Broecker**, Columbia University. **H. Brown**, Secretary of Defense, Washington, DC. **P. W. Choppin**, Rockefeller University. **R. K. Clayton**, Cornell University. **E. F. Colson**, University of California at Berkeley. **J. M. Dawson**, University of California at Los Angeles. **G. Debreu**, University of California at Berkeley. **T. O. Diener**, Agricultural Research Service, US Department of Agriculture. **S. Epstein**, California Institute of Technology. **H. E. Evans**, Colorado State University. **W. Feit**, Yale University. **S. L. Glashow**, Harvard University. **G. Goldhaber**, University of California at Berkeley. **J. E. Gunn**, California Institute of Technology. **N. B. Hannay**, Bell Laboratories, Inc., Murray Hill, New Jersey. **D. Harker**, State University of New York, Buffalo. **W. R. Hewlett**, Hewlett-Packard Company, Palo Alto, California. **G. H. Hitchings**, Burroughs Wellcome Fund Research Laboratories, Research Triangle Park, North Carolina. **L. N. Howard**, Massachusetts Institute of Technology. **J. D. Jennings**,

Person to Person

Exchange large two bedroomed centrally heated furnished apartment in Paris suburbs for one in London area from mid July to mid August '77. Car exchange also. Contact Dr Agarwal, 23 Rue de Champagne, A1760, 92140 Clamart, France.

Furnished five bedroom house in Villebon-sur-Yvette, near Orsay, Faculté de Sciences, Université de Paris-XI, France, offered in exchange for at least 3 bedroom house in Frankfurt-am-Main, Germany, for August 1977. Villebon is next to Orsay, where the Faculté de Sciences of the University of Paris-XI is located. It is about 20 km south-west of Paris. Please contact Dr Francisco Alvarado, Comparative Physiology, Université de Paris-XI, 91405, France, for further information.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

University of Utah. **N. Keyfitz**, Harvard University. **E. D. Kilbourne**, Mount Sinai School of Medicine. **M. J. Klein**, Yale University. **R. M. Krause**, National Institutes of Health. **I. R. Lehman**, Stanford University. **S. Lieberman**, Columbia University. **R. S. Lindzen**, Harvard University. **J. W. Littlefield**, The Johns Hopkins University. **H. O'Neill McDavitt**, Stanford University Hospital. **A. A. Moscona**, University of Chicago. **E. F. Neufeld**, National Institutes of Health. **O. E. Neugebauer**, Brown University. **R. M. Noyes**, University of Oregon. **S. L. Palay**, Harvard Medical School. **R. P. Perry**, University of Pennsylvania. **J. C. Phillips**, Bell Laboratories, Inc., Murray Hill, New Jersey. **G. W. Preston III**, Hale Observatories, Pasadena, California. **W. van Orman Quine**, Harvard University. **P. H. Raven**, Missouri Botanical Garden, St Louis. **C. N. Reilly**, University of North Carolina. **H. Reiss**, University of California at Los Angeles. **B. Richter**, Stanford Linear Accelerator Center, Stanford University. **R. Sager**, Harvard Medical School. **L. H. Sarett**,

Merck Sharp and Dohme Research Laboratories, Rahway, New Jersey. **R. N. Shepard**, Stanford University. **P. S. Skell**, Pennsylvania State University. **D. Slepian**, Bell Laboratories, Inc., Murray Hill, New Jersey. **H. Tabor**, National Institutes of Health. **J. H. Taylor**, Florida State University. **R. F. Thompson**, University of California at Irvine. **G. R. Tilton**, University of California at Santa Barbara. **S. C. C. Ting**, Massachusetts Institute of Technology. **W. N. Valentine**, University of California at Los Angeles. **E. M. Witkin**, Douglass College, Rutgers University. **G. N. Wogan**, Massachusetts Institute of Technology. **J. Wolpert**, Princeton University. **H. E. Wright, Jr**, University of Minnesota, Minneapolis.

Awards

The Ciba-Geigy Drew Award in Biomedical Research (\$2,000) will be granted annually. The topic for the initial awards and symposium is Viral Etiology of Cancer and the recipients are Robert C. Gallo, and Fred Rapp. The first symposium will be held on 20 October 1977.

The André Lichtwitz prize (9,500 French francs) is awarded every year by INSERM for French or foreign scientists (or a team) who have specially distinguished themselves in research on calcium and phosphorus metabolism carried out during the previous year, whether in the clinical or more fundamental fields. Conditions of entry for the 1977 prize are obtained from INSERM. Applications must reach the Director of the INSERM (c/o Mlle. C. Chirol, 101 rue de Tolbiac, 75645-Paris Cedex 13, France) by 30 June 1977.

The 'Life Institute' prize (EDF Foundation), of 300,000 F, was shared by a Japanese research team represented by Makio Ushida, and a Swedish chemist, Sören Jensen. The international jury, rewarded the Japanese for their work on the toxicity of methyl mercury, which is the cause of Minamata Disease. Sören Jensen was rewarded for his work on the polychlorobiphenyls. These organic derivatives are used in paints, lubricants, rubbers and so on. As they are fat-soluble and are not biodegradable they tend to accumulate and may contaminate food processing plants.

Vacancies

Two vacancies exist in the International Commission on Zoological Nomenclature owing to resignations. Nominations for candidates for election to the vacancies should be sent to the Secretary, International Commission on Zoological Nomenclature, c/o British Museum (Natural History), Cromwell Road, London SW7 5BD, United Kingdom, within three months of the date of publication of this notice in the *Bulletin of Zoological Nomenclature*. Candidates must be eminent scientists, irrespective of nationality, with a distinguished record in any branch of zoology, who are known to have an interest in zoological nomenclature.

Nominations must state the name, date of birth, nationality, field(s) of specialisation and qualifications of each candidate, and the name(s) and status of the nominator(s). A list of the candidate's publications and his *curriculum vitae* would also be helpful.

Reports and Publications

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- CERN—European Organization for Nuclear Research. CERN 77-05: A New Warning System for Fires of Electrical Origin. By A. H. Pietersen. Pp. v+5. (Geneva: CERN, 1977.) [283]
- Smithsonian Contributions to Paleobiology, No. 30: Triassic Echinoids. By Porter M. Kier. Pp. iv+88. (Washington, DC: Smithsonian Institution Press, 1977. For sale by US Government Printing Office.) [283]
- Solid State Physics in the People's Republic of China. Edited by Anne Fitzgerald and Charles P. Slichter. (A Trip Report of the American Solid State Physics Delegation. CSCPRC Report No. 1.) Pp. xiii+203. (Washington, DC: National Academy of Sciences, 1976) \$10.25. [283]
- New Zealand: Department of Scientific and Industrial Research. Botany Division Triennial Report, 1972/1975. Pp. 84. (Christchurch: Department of Scientific and Industrial Research, 1976.) [283]
- Institutt for Atomenergi. Kjeller. Kjeller Report 154: Atmospheric Deposition of Trace Elements in Norway Studies by Means of Moss Analysis. By E. Steiness. Pp. 13+3 tables+10 figures. (Kjeller, Norway: Institutt for Atomenergi, 1977.) [283]
- Tracor Inc. 1976 Annual Report. Pp. 32. (Austin, Texas: Tracor, Inc., 1977.) [293]
- European Brewery Convention. Reports of the Barley Committee. Field Trials 1973 (Malting 1974). Pp. viii+155. Field Trials 1974 (Malting 1975). Pp. viii+160. Field Trials 1975 (Malting 1976). Pp. viii+163. (Vols. 24, 25 and 26.) (8050 Freising-Weihenstephan, West Germany: L. Reiner, EBC-Databank, Techn. Univ. München-Ackerbau und Versuchswesen, 1974, 1975 and 1976.) [303]
- Bulletin of the American Museum of Natural History. Vol. 158, Article 3: A Synopsis of Asian Species of *Mus* (Rodentia, Muridae). By Joe T. Marshall, Jr. Pp. 173-220. (New York: American Museum of Natural History, 1977.) \$2.90. [303]
- Climate and Food: Climatic Fluctuation and U.S. Agricultural Production. Pp. ix+212. (Washington, DC: National Academy of Sciences, 1976.) \$7.75. [303]
- World Health Organization. Technical Report Series, No. 602: Advances in Viral Hepatitis—Report of the WHO Expert Committee on Viral Hepatitis. Pp. 62. (Geneva: WHO; London: HMSO, 1977.) \$r.fr.8; \$3.20. [313]
- United States Department of the Interior: Geological Survey. Professional Paper 971: Structural Geology of the Confusion Range, West-Central Utah. By Richard K. Hose. Pp. iii+9+plate 1. (Washington, DC: US Government Printing Office, 1977.) [313]
- CERN—European Organization for Nuclear Research. CERN 77-04: The $\pi\pi$ Interaction. By J. L. Petersen. Pp. 109. (Geneva: CERN, 1977.) [313]

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- Nonlinear Analysis: Theory, Methods and Applications, Vol. 1, No. 1, 1976. Edited by V. Lakshmikantham. Pp. 1-90. Annual Subscription Rates for 1977: For libraries, University Departments, Government Laboratories, Industrial and other multiple-reader institutions US\$8. Individual who institution takes out a library subscription may purchase a second or additional subscriptions for personal use US\$30. (Oxford and New York: Pergamon Press, 1977.) [44]
- Monitoring and Assessment Research Centre, Chelsea College, University of London. MARC Report No. 1: The Ozone Depletion Problem (An example of

Harm Commitment). By Lester Machta. (A General Report.) Pp. 33. £1; \$2. MARC Report No. 2: Vanadium in the Environment. By Siv Bengtsson and Germund Tyler. (A Technical Report.) Pp. 17. £1; \$2. MARC Report No. 3: Suggestions for the Development of a Hazard Evaluation Procedure for Potentially Toxic Chemicals. By Robert C. Harriss. (A Research Memorandum.) Pp. 18. £1; \$2. (London: Monitoring and Assessment Research Centre, Chelsea College, 1977.) [44]

Advances in Extractive Metallurgy. (An International Symposium organized by the Institution of Mining and Metallurgy and held in London from 18 to 20 April, 1977.) Pp. vii+244. (London: The Institution of Mining and Metallurgy, 44 Portland Place, W1, 1977.) £18.50. [44]

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The National Health System in Denmark: a Descriptive Analysis. By D. Gannik, E. Holst and M. Wagner. (Publication of The John E. Fogarty International Center for Advanced Study in the Health Sciences. US Department of Health, Education and Welfare, Public Health Service, National Institutes of Health.) Pp. vi+86. (Washington, DC: US Government Printing Office, 1977.) \$1.15. [124]

United States Department of the Interior: Geological Survey. Bulletin 1420: Mineral Resources of the La Garita Wilderness, San Juan Mountains, Southwestern Colorado. By Thomas A. Steven and C. L. Bieniewski. (Studies Related to Wilderness—Wilderness Areas.) Pp. vi+65+1 plates. Professional Paper 989: Plutonism and Orogeny in North-Central Washington-Timming and Regional Context. By Kenneth F. Fox, Jr., C. Dean Rinehart and Joan C. Engels. Pp. iii+27. (Washington, DC: US Government Printing Office, 1977.) [124]

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nature

2 June 1977

Technology transfer at DOI

THE latest Report on Research and Development from Britain's Department of Industry (DOI), for the two years 1974–1976 (HMSO; £1.25), comes just at the time that Sir Ieuan Maddock hands over the post of Chief Scientist in the Department to Dr Duncan Davies, who was until recently in overall control of research at ICI. Research and development at DOI is not about to undergo sweeping changes as a result of this move, as the strategy for the next few years is bound to be evolutionary, but the report is valuable as marking more than just the end of one man's chieftainship—the Rothschild reorganisation of governmental support for science was also completed in the period under review, so it is possible to look at the whole new policy-making structure erected in the past few years.

The department now spends roughly £100 million per year in industrial research and development, and of this about one-half goes into DOI research establishments (which include the National Physical and National Engineering Laboratories), and other governmental organisations. The remainder goes to industry, either directly (largely to the aerospace industry for the reduction of fuel consumption and noise) or indirectly by way of Britain's subscription to the European Space Agency of which DOI pays the major part. Away from the aerospace field, expenditure is widely scattered over subjects ranging from quantum metrology and waste recovery to cloth cutting for the garment industry. Research is guided by means of eight requirements boards, the membership of which is roughly half industrial, half academic and governmental.

The research and development aspect of the work is obvious and it is DOI's expressed intention to place more of its contracts out with industry itself, which should be welcome news for the not-exactly-buoyant industrial research sector. It should also place the governmental research establishments firmly on their

mettle to adopt aggressive programmes of their own, reaching out, as did Harwell several years ago with some success, in the hope of tapping new sources of funding. This will be a long haul. It is clear, however, that as the requirements boards begin to develop greater selectivity and acquire greater influence, they will want to avoid any pretensions that they act in some way as a sort of proxy management for the research establishments.

The other major aspect of DOI's task is to transfer technology out of the laboratory and into the factory. It has recently been a departmental theme that the national emphasis on aerospace has been to the detriment of the low-technology or small industry where the largely unmet demand is for modern rather than highly advanced technology. And it is in this field of the application of already existing technology that very interesting second careers are to be made.

We have repeated almost *ad nauseam* that the civil-servant scientist and technologist finds him or herself in an unfortunate bind in that seniority in the service leads, for most, not to more broad administrative and managerial responsibilities but to more of the same thing, often against a background of rapid and difficult-to-absorb change. In the field of technology transfer there could well be scope for the gainful employment of many who are at the present doing little more than marking time. For the skill that is most needed is not intellectual brilliance but wide experience. To be sure, not every one-time scientist or technologist is going to be able to develop into an ideas broker, capable of getting, in the most unabrasive way, into the textile mills or the workshops under the railway arches to hear about old problems and to talk about new techniques. But there must be some who would relish the challenge of such a job. Maybe DOI's new Chief Scientist will be tossing such an idea into the laboratories under his wing. □

Séveso solicitude

Alastair Hay visits Séveso and finds political as well as technical problems

IT IS ten months since the ICMESA chemical plant reactor exploded discharging tetrachlorodibenzo-*p*-dioxin (dioxin) over a large section of the Italian town of Séveso. Though considerable progress in the reclamation of the largest residential portion of the area is now apparent, the record of success has not extended to actual physical destruction of the dioxin present in the contaminated material collected. Vacillation over the methods to be adopted is caused by several factors: a paucity of relevant scientific data, hostility by Séveso residents to some of the methods which the Lombardy regional government has proposed, and indecision in government circles at the regional and national level. At regional level the preferred course of action seems to be destruction by incineration, but Séveso residents view construction of the incinerator with alarm and this could prove electorally damaging to the political parties concerned.

Professor Augusto Giovanardi, coordinator of the Decontamination Commission appointed by the Lombardy regional government to supervise operations at Séveso, says that according to measurements made by the commission between 300 and 500 grams of dioxin were deposited in the most contaminated area (designated zone A) and that some 10 grams were present in the less affected zone B. He dismisses the suggestion made by a British scientist, Dr Donald Lee, that up to 130 kg of dioxin could have been produced in the reactor explosion. However, Giovanardi acknowledges that neither his commission nor Givaudan had been given permission by the Italian courts to check the reactor contents. This analysis was being carried out solely by scientists employed by the judiciary.

Seven sections

For the purpose of decontamination policy zone A has been divided into seven sections. Areas A1-A3—those nearest the reactor—are the most seriously contaminated, and contain about 80% of the discharged dioxin. A4 and A5 are areas of 'medium contamination'; A6 and A7, the least affected portions of zone A, housed 80% of the residents evacuated from the area by the local authority. Consequently it has been sections A6 and A7 which have received most attention by the authorities. Giovanardi explains

that buildings in the area have been decontaminated by a process of vacuum cleaning and washing with solvents. "Most of the soil and vegetation from the area had been stripped away two weeks ago", he says, "and the pavements and roads will be the last portions to be cleared". He adds: "We hope that the people with homes in the area will be able to return to them sometime in the months of June to August this year".

Concurrent with the decontamination work in zone A the commission has been working in zone B, which may yet have to be expanded. Here the residents have been asked to abide by certain rules to minimise personal exposure to dioxin. Children leave the area for the daylight hours, and women in the first trimester of pregnancy are advised to move from the district until the foetus is at least three months of age. With the work on sections A6 and A7 now nearing completion Giovanardi says the commission "is now applying the same operation to the houses, vegetable gardens and so on in zone B that we have used in A6 and A7". Contaminated soil and vegetation from these areas is being deposited in the 'medium contaminated' sections A4 and A5. The vegetation is to be incinerated, but the soil may well be left unprocessed in the hope that microbial degradation will reduce the dioxin concentrations to acceptable levels.

Whereas the consensus of scientific opinion is that the soil should be removed from zones A6 and A7 and definitely from the 'heavily contaminated' areas A1-A3, opinions differ on the methods to be applied to degrade the dioxin present. Incineration is apparently the approach favoured on theoretical grounds by some members of the commission, but this view is by no means universal. Other scientists are conducting trials on photochemical decomposition of dioxin, solvent extraction of the chemical (from the soil) and degradation by steam distillation.

The most vehement opposition to the incineration proposal and to the plans to construct an incinerator on the site of the ICMESA plant comes from the Séveso residents. They have had enough. If the incinerator has to be built, they argue that it should be built somewhere else. They know that incineration of the soil on the scale proposed will be an enormous under-



Barrier to progress?

taking. It will be an untried process and they claim that they are to be the 'guinea pigs' of the regional authority. In the past month they have mounted a demonstration in Milan to protest against the incinerator's construction and gunmen have broken into the office of the health officer at Séveso, shooting him several times in the legs.

Information relevant to the handling of dioxin-contaminated soil has been procured for the Italian authorities by Professor Barry Commoner from the US Department of Defense. On a visit to Italy in February and March, Commoner expressed concern and disappointment at the measures being adopted. He wrote that it was evident that "a systematic examination of alternatives [for degrading dioxin] has not been carried out" by the Decontamination Commission, and much of the decontamination work was "unnecessarily confused". Asked about Commoner's criticisms, Giovanardi's response is that whereas incineration appears to be the most feasible degradative process, his commission is considering all alternative procedures.

Government Commission

Last year the Italian Government appointed its own Commission to assist the local authorities at Séveso and the regional government in Lombardy. The Under Secretary of State for Health, Dr Fernando Russo, is the commission's president. As such he has the responsibility for collating all the available information on dioxin both nationally and internationally. He says there is "no evidence available on the complete destruction of dioxin. We are still searching for this evidence on an international scale".

Russo says that the final aim of all the authorities is to reduce the dioxin

levels to zero. The Ministry of Health convened a meeting in Rome recently to assess the situation at which a whole series of programmes were discussed. Many problems remained unsolved. Séveso has been a very bitter lesson for Italy. It has forced the government to review its legislation on industrial hazards, pollution control, toxicology and food additives, amongst other things. The message has been rammed home that 'prevention is better than cure'. Russo says Italy would like legislation on pollution control in particular to be a European Community measure, for national legislation on this issue "might provoke reprisals" and force multinational companies to go elsewhere.

As for the specific pollution problem at Séveso, Russo is in no doubt that ICMESA's owners Givaudan, as a big company, "will more or less have to pay" for the damage they caused, estimated now to cost 113 billion lira—about £80 million. Givaudan states that this figure seems "extremely large".

Just to ensure that they do receive payment, the local authorities in and around Séveso, the Lombardy regional government and the central government are all involved in litigation for damages against Givaudan. The company, they claim, had inadequate safety features operating in the trichlorophenol reactor. And to compound the problem, when the accident occurred the ICMESA management provided insufficient information on the pollution hazard. As a result many people were put to unnecessary risk by their exposure to dioxin for the two weeks before residents were evacuated.

Central matter

This two week period of exposure is central to the health problems of the Séveso population and to the issue of

abortion for the pregnant women exposed to dioxin. Although Russo claims that the Italian government wasn't against abortion for these women and that the opposition came from local doctors at Séveso, he has the impression that it hadn't been too difficult for the women to procure legal terminations.

This impression is not confirmed by Ms Adele Faccio, the Radical Party Member of Parliament for the constituency of Genoa. She was in the Séveso area in July and August last year and, as a result, has been particularly concerned about the question of abortions for the Séveso women.

"It was extremely difficult for these women to obtain legal terminations", she declares emphatically. To substantiate her claim she cites the figures for the abortions performed on the women. "Only 30 women had legal terminations", she says, "whereas twice this number have had illegal abortions. The illegal terminations were performed by 'women's self-help groups' for cases where the foetus was not older than ten weeks. For women in a more advanced state of pregnancy it was essential to send them abroad for an abortion".

But if legal abortions have proved difficult for the Séveso women, there is some relief in Italian medical circles over the results of the examinations performed on the foetuses from those women permitted legal terminations. These examinations were conducted by Professor Alfred Gropp and Dr Helga Rehder of the Lubeck Medical School, Germany, and Professor Francesco Céfis and Dr Laura Sanchioni of the Istituti Clinici di Perfezionamento in Milan. Of the 34 foetuses examined only one showed any signs of abnormal development. As with most of the foetuses examined, however, this one embryo was badly macerated—a common situation with induced abortions.

The results must therefore be interpreted with great caution. They could be suggestive in this one instance of foetal Down's syndrome. This, however, is a not uncommon foetal abnormality in most populations. Some UK clinicians think it is unlikely to be due to dioxin. Professor Gropp and his colleagues claim in a report to the Lombardy Regional Health Authority that "the results of the embryo-morphological studies do not give any hint for damage brought about by the action of exogenous agents (dioxin)". They add the rider, however, that the negative result must not necessarily mean that dioxin "does not bear a risk for the mother and foetus". The examinations had certain inherent limitations: minor malformations and particularly organ damage

are not detectable in the very young foetus; only a limited number of cases could be studied, and most of these embryos were incomplete and of different gestational age.

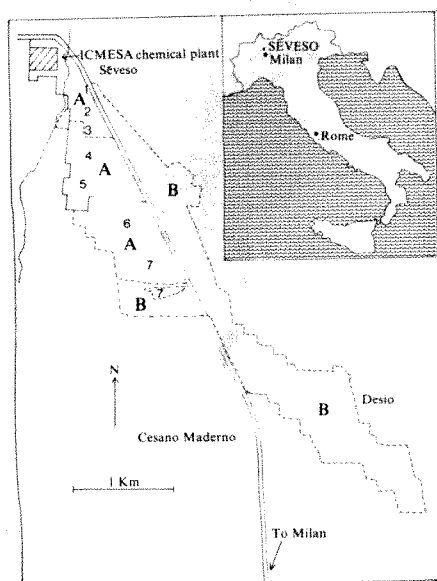
Professor Gaetano Fara, chairman of the Lombardy region's Health and Epidemiology Commission for Séveso, adds a further qualification to the interpretation of these results. He admits the foetal examinations cannot be interpreted in the context of a true epidemiological survey; they are an analysis of data to hand. He adds that, for a true epidemiological survey, the population would obviously have been much larger and more carefully monitored.

As for the eight children from the Séveso area born with abnormalities, Fara claims that statistics would indicate that this incidence of malformation is no greater than the average for the region. Some epidemiologists in the UK have expressed the opinion that because of the variation in the pattern malformations reported at Séveso it is quite possible that they are not due to dioxin. They add that, if the chemical was responsible for these abnormalities it would be reasonable to expect a more consistent pattern in the defects observed.

Even though some of the medical results appear to be reassuring, Fara insists that they still consider the Séveso residents to be a 'population at risk'. He said that a preliminary report on the children from Séveso based on three visits to doctors had revealed no immunological damage in this group, compared with their peers in a non-contaminated area. Similarly chromosomal studies performed on the 34 aborted foetuses by Dr Luigi de Carli at the University of Bafia had revealed no genetical deviations from the accepted norm.

But Fara does admit that the cases of chloracne reported represent an area greater than that officially designated as being contaminated. He acknowledges that it is worrying, but adds that of the 525 cases of reported chloracne, only 1 in 4 were confirmed on subsequent re-examination. In view of the toxicity of dioxin, however, the regional authority is to embark on a large scale epidemiological study of the area. Fara says that the population will have to be monitored for years to come.

If Professor Giovanardi's predictions are correct the Decontamination Commission will not complete its programme to bring dioxin down to an acceptable level before 1980. By this time most of the residents will have returned to their homes, but as a population still 'at risk' fears about dioxin will still be a part of their lives. □



Scientists and education in Poland

Social change in Poland is affecting the country's science and education policies. A **Special Correspondent** reports

DURING periods of heightened social tension, general issues dominate science and education policy, as the present situation in Poland illustrates. The main problems which face the country are political rather than economic. The beginning of a wave of repression against the participants in strikes in June 1976 (*Nature*, 5 August 1976) followed the abortive decision to raise prices significantly. On 23 September 1976 the Workers Defence Committee (KOR) was formed by a small group of courageous people. It made several demands; unconditional amnesty for all sentenced for their participation in strikes and demonstrations; provision of appropriate jobs for all workers dismissed from the factories and restoration of long-service privileges; disclosures of all repressions including beating by the police while in custody, false accusations and confessions coerced by force, unlawful conduct of courts and so on; and the setting up of an independent parliamentary committee to investigate all facts related to infringement of human rights.

Acting openly in spite of the fact that it is illegal, the KOR, consisting of 23 members, enjoys considerable support. It is collaborating with a fairly wide group, mainly students and recent graduates drawn from participants in the student riots of March 1968 and from the Clubs of Catholic Intelligentsia and, to a lesser degree, from the Scouting organisation. It is their devoted work that enables KOR to collect and distribute money (about 500,000 zlotys up to the end of March); to gain access to the people involved

and to monitor the court proceedings; and to publish and circulate KOR's *Bulletin* (nine issues so far) and other documents. KOR's appeal for public support has been answered by many letters of protest to the Polish Parliament, from more than 2,500 people. They include 730 students from the University of Warsaw, 520 from the University of Krakow, and 285 from the Catholic University in Lublin.

KOR is in no sense a political party: it has no political programme and the political opinions of its members and collaborators differ considerably. None of its activities is directly political, and this may be the source of its strength and early immunity. The tactics chosen by KOR have been effective; its very existence has structured internal political life and provided a new pattern of defence of civic rights under the communist regime. This is manifested in the growing self-assertion of diverse social groups in exercising their rights under existing laws. Writers have produced a first unofficial literary journal *Zapis*, a large type-written volume of poetry, stories and criticism. A number of political programmes and groups have appeared, some of them critical of KOR.

Scientists have also been active. Thirty-four professors, most of whom have not raised their voice in public before, sent a letter of protest to Parliament. They included many well-known scientists and members of the Polish Academy of Sciences (PAS): W. Kemula (Chemistry), W. Miemierko, J. Kielanowski, W. Kunicki-Goldfinger, E. Domanski, A. Brzoza, M. Nunberg

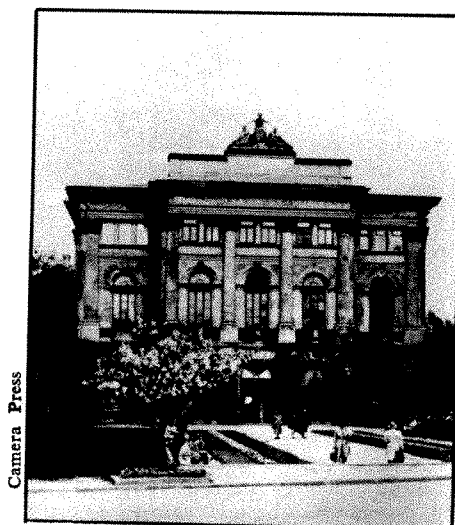
(Natural Sciences), I. Chmielewska (Biochemistry). Another letter was signed by about 300 younger scientists and artists. Although these letters are the most visible protestations, others are no less significant. During the General Assembly of the PAS on 17 December 1976, for example, several members of the Academy opposed the established procedure which reduces the role of the Assembly to a purely formal acceptance of candidates proposed by the Praesidium (which in turn is completely dependent on the political authorities). Three recommended candidates were not elected. Among them were Professor J. Szuba (favoured personally by Mr Gierek, the Secretary of the Party) and Professor Z. Rybicki, the Chancellor of Warsaw University.

Teachers' protest

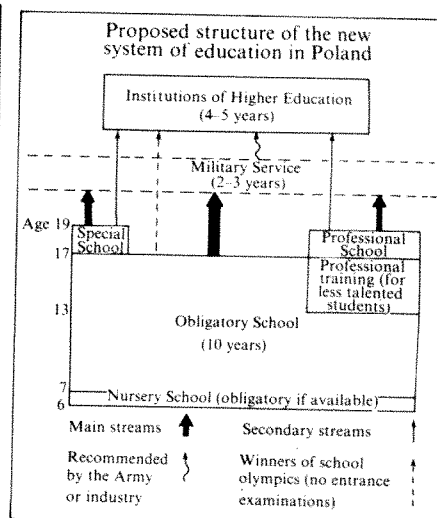
Some letters of protest were signed by school teachers. In Poland the teaching profession is particularly weak because for many years it absorbed weaker graduates unable to find jobs of higher social standing and independence from administrative bodies; also the teaching profession consists almost entirely of women. The teachers' publicly expressed protests were followed immediately by repressions. In the Tadeusz Rejtan secondary school in Warsaw, for example, six teachers were given dismissal notices (three of them are to be transferred to other schools, and three have been asked to retire early; these decisions have not yet been implemented). Ironically Tadeusz Rejtan was the member of Parliament who fought against the first partition of Poland in 1773. The action against teachers immediately provoked a new flow of protests, this time from the school's students and graduates.

No similar case of repression of older members of the scientific community is known to this correspondent,

Miloslaw Chojecki, a nuclear physicist, and Piotr Naimski, a biochemist, were recently arrested as part of a new campaign of the Polish Government against the Workers' Defence Committee. In all, some 25 activists were arrested, apparently as a preventive measure. The recent death, in mysterious circumstances, of Krakow student Stanislaw Pyjas has led to renewed agitation against alleged police illegalities, and it is thought that the arrest of the activists was intended to neutralise this opposition. In spite of the arrests, however, the agitation, and demands for a public inquiry into the death of Pyjas, are still increasing.



Warsaw University library



and the authorities try not to create martyrs. The situation for younger scientists, however, is different. The case of Mr Chojecki, who was dismissed from the Institute of Nuclear Research, has already been mentioned in *Nature*. More recently Mr J. Celinski, who was dismissed from the University of Warsaw, brought his case to court. The court upheld the decision of the university because, as formally stated in the judge's justification,

Each department and its chief is responsible for the results of work and is free to choose employees with appropriate professional qualifications. These qualifications include sometimes . . . political criteria . . . and the political convictions of the plaintiff disqualify him from being a member of staff of the University of Warsaw.

These actions against teachers and scientists are of course relatively mild. Under present international circumstances, however, it is precisely the activity of KOR that makes the escalation of repression difficult. Each country has its own scale of values and measures of tolerance which form an ethical frame in which the political repressions in Poland should be judged.

Another major determinant of the situation is the plan for a new educational system. Parliament passed a curious bill describing the general shape of future changes but without specific decisions. The ten-year school, the basis of the new system (see figure), is not a secondary school in the ordinary sense, because entry to the university is guaranteed only for the winner of school olympics (a special type of competitive examination) and for those who have a record of "two years of excellent professional work or two years of impeccable military service".

The most striking feature of the new system is that most future students will have to complete military service before entering university. However, there will be other options for students leaving the ten-year school, ranging from six-month professional courses to two years of further general education. One can expect therefore a highly differentiated level of readiness for university studies which must impinge on the performance of the universities. The further feminisation of the universities would also follow. The fraction of women in institutions of higher education is about 52%; arts faculties have 77%; education 74%; medicine, with some measure of preference for men, has 64%; engineering, the least feminised faculty, has 34%.

Implementing the plan

Preliminary steps for introduction of the new educational system have

already been taken. About 70,000 acting teachers are being taught in special schools or special faculties of universities. It is commonly accepted that the courses they offer are poor, but they are entitled to confer masters degrees.

No one can deny that the present schooling system has serious shortcomings and does not serve the social needs of Poland. However, it is also true that discussion of the new system was deliberately channelled on to such problems as curricula, teacher training, availability of buildings and so on, and was made to bypass the evident political motives underlying the reform. The first of these is the failure of the schools' ideological influence; both teachers and students have adopted a sort of newspeak understood by both sides to be meaningless. Second, the schools do not provide appropriate political filters; in spite of some incentives, the number of students from workers' and farmers' families is not increasing and these students are in no sense more loyal to the party than other students. The authorities hope that the new system will provide more effective social indoctrination and more reliable political filters.

It is still unclear when implementation of the new system (planned for 1978) will begin. The system is very costly and its social price—the loss of experience accumulated by the existing system—is even higher. Postwar social changes in Poland opened the possibility of study for all who desired it, so making possible the social promotion of all gifted people from the working class. The byproduct of this process, however, was a significant decrease of the working class's own intelligentsia, a group which contributed most to the 'striving for education' within its class. Those gaining education often break their ties with their previous social environment (formally too they are not considered as members of the working class any longer) and constitute a very conservative group of people who, as they see it, have nothing to win and everything to lose.

There are additional reasons for the declining attractiveness of university studies for young people from the working class. Nowadays students who cannot rely on their families for support are in a worse position than their colleagues in the 1950s, because of a decrease in the real value of scholarships. More importantly, graduate prospects are not very bright: it is not easy to find appropriate jobs, salaries are relatively low, and there are serious difficulties in getting a flat (formally graduates have a high priority in the queue for flats, but the overall short-



The Palace of Culture, seat of PAS

age makes this privilege of negligible value). Usually the new graduate is worse off than his less gifted friend from the primary school.

The political control of universities is exercised primarily by the careful selection of staff. No one can be engaged without the consent of the party organisation and without a good record from the police. The admission to post-graduate studies is similarly controlled, and several people who pass the examination are not admitted because of their or even their parents' political attitudes. In the recent enactment of the Ministry of Science and Higher Education (December 1976) it is openly stated that to be admitted to post-graduate studies the candidate has to "display proper ideological and civic attitudes". The chosen faculty has little relevance in this regard and candidates in social sciences and, say, medicine are treated on the same basis.

The fate of the new movements which are changing considerably the pattern of internal politics in Poland and, more generally, the immediate future of this country, are closely related to the whole human rights issue. This makes the proceedings and the results of the Belgrade Conference vitally important for Poland. At best one can expect some softening of the political line, and a more elastic and realistic approach to the needs and demands of people which could lead to a better economic performance. At worst one can foresee a partial return to cold-war policy with the rise of police power and a further widening of the gap between the rulers and the ruled. But even in this case the experience gained during the last few months could play an important role: Polish society has learned a lesson which it will be difficult to erase. □

USA

The threat to academic science

Colin Norman reports from Washington on a study of the state of US academic research

ACADEMIC scientists in the United States have been complaining bitterly during the past few years. Declining budgets and increasing amounts of red tape are putting severe strains on the country's research system and gradually eroding the quality of basic research, they claim. Yet American science remains outstanding by any measure: total funds for research and development are expected to reach about \$41,000 million this year, and scientists from the United States walked off with every Nobel Prize awarded last year. So do the complaints have any real basis, or are they simply self-interested pleas for more money?

According to an impressively detailed and closely argued report published this week*, there is indeed plenty of cause for concern about the health of academic research in the United States. Written by two social scientists working with a grant from the National Science Foundation (NSF), the report reaches this ominous conclusion: "The discoveries that are now being made are based on research initiated many years ago, the system is operating on momentum, but there are disturbing signs that the momentum generated during World War II and for 25 years thereafter has been halted. An important national resource may be eroding and the nation is faced with the possibility that it will lose scientific and technological leadership in many fields".

The authors of the study, Bruce L. R. Smith of Columbia University and Joseph R. Karlesky of Franklin and Marshall College in Pennsylvania, base that lugubrious assessment on a number of factors which are hurting the universities in general and university science in particular. Their chief message is not that academic science is in imminent danger of collapse, but that a number of far-reaching changes are taking place in the research system. "Failure to deal adequately with the long-term adverse trends could generate a decline from which recovery will be difficult and protracted", they warn.

In particular, Smith and Karlesky suggest that the problems afflicting university research could lead to

greater stratification in the higher education system, with fewer universities conducting research of the highest quality. There could also be a weakening of the traditionally close links between research and teaching, and funding constraints could lead to less support for innovative and speculative research, they warn. But the potential changes are not all bad, for Smith and Karlesky note that in 1967 the between universities and industry, after a considerable period of arms-length relationships has begun to take root again".

The most obvious problem affecting academic research is that federal support for research and development abruptly shifted gear in the late 1960s, moving from a period of burgeoning growth to a period in which total funding failed to keep pace with inflation. Smith and Karlesky cite figures, for example, which indicate that federal expenditures on basic research dropped by about 15% in constant dollars between 1968 and 1976. And that abrupt shift coincided with a turn for the worse in the general financial health of the universities as many sources of public and private funds began to dry up while overhead costs began to increase by leaps and bounds.

Superimposed on that trend of shrinking total support has been a marked change in the nature of federal funding for academic science. Smith and Karlesky note that in 1967, the federal government spent \$1,301 million to support specific research projects in colleges and universities, but another \$1,022 million was provided in the form of general research support (block grants to institutions, graduate traineeships and grants for improving general scientific capabilities). But by 1975, that pattern had changed dramatically: support for specific projects had increased to \$2,222 million while general research support had dropped to only \$567 million. "This substantial decline in institutional funding means that universities have at their disposal less discretionary money to fill gaps, start new exploratory studies . . . and meet other special and changing needs". In short, universities have lost considerable flexibility in the use of federal research funds.

A major impact of the decline in financial support has been a marked deterioration in the quality of scientific instruments and other laboratory equipment in the universities, as "a number of universities have postponed the replacement of equipment and deferred maintenance", Smith and Karlesky

suggest. They cite, for example, the case of one public university in the midwest which has been unable to provide any funds for scientific instruments for the past six years, and they suggest that the problem of replacing outdated instruments and poor facilities is now facing even the most prestigious universities.

It should be noted, however, that the NSF budget for fiscal year 1978, proposed by President Ford shortly before he left office, includes some \$38 million for upgrading instruments in university laboratories. The NSF budget is, however, encountering some difficulties in Congress, and it is by no means certain that even that small relief measure will be fully approved.

A second major factor affecting the health of university research has been a marked deterioration in the academic job market for young scientists, as university expansion abruptly ceased in the early 1970s and the proportion of faculty members with tenure has increased sharply. Though there are significant differences between universities and between different fields of science, Smith and Karlesky report that the proportion of faculty members occupying tenured positions increased from 47% in 1969 to 65% in 1973. Moreover, according to a survey conducted by NSF, the tenure ratio in 1974 varied from a low of 59.1% in physiology to a high of 80.7% in chemical engineering.

"There is a growing uneasiness that the career pipelines may be too restricted and that determinants other than excellence are increasingly controlling career patterns", Smith and Karlesky report. They suggest, moreover, that "the decline in research opportunities for young investigators will be one of the most serious threats to the momentum of university research over the next decade". One noteworthy impact of the tenure logjam is, in fact, already showing up: the proportion of young faculty members (those who have held a PhD for less than seven years) declined from 43% in 1968 to 27% in 1975, and a further decline is anticipated by 1980.

As for enrolments in graduate departments, there was a major drop in the physical sciences and engineering in the early 1970s, while enrolments in the life sciences and the social sciences continued to increase. In some institutions, Smith and Karlesky predict that the rapidly shrinking numbers of graduate students could lead to decisions to drop some PhD programmes, a move which would force a painful choice between continuing to mount a research effort in the absence of graduate students or reducing re-

**The State of Academic Science* (Change Magazine Press, New Rochelle, New York; \$5.95).

search efforts to concentrate on teaching undergraduates. Such a decoupling of research and teaching functions would represent a significant departure from the traditional university role, but Smith and Karlesky note that some university departments are already contemplating whether to continue as research centres.

One of the major findings of the study was that recent trends in funding and enrolments have been felt most keenly by less distinguished departments and by less distinguished investigators. "In contrast", Smith and Karlesky report, "the leading science departments have tended to retain their relative strength . . . the downward trend has not appeared uniformly throughout all second-rank institutions, and the indicators of trouble have not always reached an advanced state. But the signs are sufficiently clear to warrant the conclusion that a very rapid deterioration in the relative position of many weaker departments could well occur in the near future".

In a sense, Smith and Karlesky note, it is "highly reassuring that our system of awarding research grants and contracts according to scientific merit appears to have achieved its aim in making certain that pre-eminent investigators are able to continue their work, and in most cases with adequate support". Nevertheless, the increased stratification in university research departments and the possibility that some second-rank institutions will drop research all together, raises a number of fundamental questions.

Although PhD programmes probably over expanded during the 1960s and "some of the research undertaken a decade ago was of less than first-rank quality", how far should the drift toward fewer and fewer outstanding research departments be allowed to proceed? Smith and Karlesky argue that "it is possible that the competitive elements in the system could be undermined if there were too few universities fully equipped for pre-eminent research. The nation would then be forced to rely on a narrow base of dominant centers".

In any case they argue that the trend "towards fewer universities and departments able to mount first-class research efforts will continue so long as federal research support remains essentially level, which is likely to be the case to some extent. Universities may be able to preserve their research missions by achieving a functional division of labor among themselves and by developing highly specialised research programs. But there is a certain minimum coverage of basic science fields that a university must undertake to retain its research vitality". □

200-mile zones: the threat to marine science

AS THE sixth session of the gruelling Law of the Sea Conference opened in New York last week, the National Academy of Sciences warned that the latest draft treaty could "cripple" marine scientific research. The Academy's warning, contained in a letter sent to Elliot Richardson, head of the US delegation to the conference, claimed that oceanographic research is in fact already being seriously hampered by restrictions imposed by some coastal nations.

The chief concern is that the draft treaty now under discussion (called the Revised Single Negotiating Text, in United Nations parlance) would give coastal states the right to regulate scientific research conducted within 200 miles of their shores. Since the coastal regions contain some of the most interesting oceanographic phenomenon, such as the major currents, virtually all of the oceans' biological activity and most of the world's undersea earthquakes, the Academy is worried that the treaty could put major areas of scientific research off-limits.

The revised single negotiating text would require that the consent of a coastal state be obtained before a research project is conducted within 200 miles of its shore. Consent could be denied for research which "bears substantially on the exploration of the living and non-living resources", involves drilling, or interferes with fishing or other economic activities. In addition, the coastal state would have the power to veto publication of research results from some types of projects, and it would have the right to halt a project if it believed that the research differed from the description given in the original proposal. Such restrictions could "cripple future marine scientific research which will be critical to the survival of the oceans and mankind", the Academy stated.

The Academy is therefore urging that the draft be modified to provide the following conditions for research projects conducted outside a nation's territorial water (which will probably be 12 miles), but inside the 200-mile 'Economic Zone':

- Freedom of research should be guaranteed except for carefully specified and limited types of projects.
- Specific criteria should determine whether or not a project requires prior

consent, and a definite procedure should be established for obtaining consent. The chief objective would be to ensure that no unreasonable or arbitrary restrictions are imposed.

- Freedom to publish and disseminate research results should be preserved.

In addition, the Academy recommends that the nation undertaking the research should be required to keep the coastal state fully informed of the nature, objective and schedule of a proposed project. The coastal state should also have a right to be represented in the project, and it should be given preliminary and final reports, a share in the data and samples, and assistance in interpreting the results.

To support its contention that the provisions contained in the draft treaty would seriously hamper research, the Academy cites evidence that a number of coastal states are already exercising strict controls over projects within 200 miles of their shores. In particular the Academy notes that "in the past year the records of the US National University Oceanic Laboratory System, which coordinates the activities of the academic fleet, indicate that about half of the scheduled cruises for work in waters over which other nations claim control have been cancelled because requests were denied, or have been hindered sufficiently to prevent the cruise taking place. Some requests were never acknowledged; sometimes approval came too late for the program to be successfully conducted. At least 18 nations were involved in one way or another in inhibiting science in this way".

In spite of the Academy's strong plea, oceanographers do not hold much hope that the final version of the Law of the Sea Treaty (if such a document ever emerges) will protect marine research from arbitrary or unreasonable restrictions. Coastal states sometimes claim that research vessels are used as a cover for military or commercial operations and their activities should therefore be tightly monitored and controlled. Moreover, since the oceanographic research fleet belongs almost exclusively to the developed countries, there's little immediate incentive for developing countries to grant freedom of research within their economic zones.

Colin Norman

USSR

● The gap between R&D and the implementation of results is a common complaint against the whole spectrum of Soviet industry. In most cases the problem is simply one of logistics, the reorganisation of production plans and the re-equipping of plants without interrupting quarterly production schedules. For high-vacuum technology, however, the problem is more complex, as there is no single body responsible for R&D in this field. According to a recent *Pravda* article, the Ministry of Chemical and Petroleum Equipment, is prepared to tolerate a "considerable" gap between production and requirements during the current five-year plan; the Ministry of the Communications Industry, which needs the results of research in this field, will not itself undertake it "considering it to be the business of other branches."

No single body or ministry, it would seem, has high-vacuum technology high on its list of priorities. It is the Cinderella of research subjects, and it is not surprising, therefore, that a report on a pilot cryogenic 1,000 kW electric generator at present under test in Leningrad speaks gloomily of the technical problems involved, although it suggests that plans for a similar generator of up to 2 GW are already in operation.

Last December, an article by G. Saksaganskii, Head of the All-Union Section of Vacuum Apparatus, suggested a way out of this problem: the establishment of a 'Scientific-Technological Union' which would be responsible for satisfying all requirements in this field, over-riding ministerial and departmental barriers. He put particular emphasis on the need for custom-built apparatus for highly specialised branches of research and industry, and praised the high levels attained in this field by foreign firms.

A recent edition of *Pravda* says that Saksaganskii's article provoked a wide response from readers in industries which have some concern with vacuum technology. All the letter-writers "unanimously" supported his proposal for a single organisation to deal with "specialised" production. But, it is claimed, the letter-writers went even further: not only does industry require a special body to ensure production, there should also be a unified authority in charge of R&D. A number of writers proposed that a special scientific council should be established in the Academy of Sciences of the USSR "to coordinate theoretical research in the field of vacuum technology and the practical implementation of their results". In

addition, steps should be taken to ensure that there are sufficient number of highly-qualified personnel in the field of vacuum technology. So far, it is said, the ministries whose sphere of competence touches upon high-vacuum technology have made no comment on these suggestions.

A press campaign of this type is a traditional precursor of a major change in Soviet policy, and the response to Saksaganskii's article seems



to reflect a genuine discontent. The Soviet press frequently carries eulogies of the benefits of centralised planning. The current state of the high-vacuum industry, is, however, a salutary warning of what may occur when a particular branch or field of development is somehow omitted or overlooked, if not in the plan itself then in the practical details of implementation.

● The Byelorussian SSR is traditionally one of the poorest areas in natural resources of the whole of the Soviet Union. When, some 20 years ago, a large oil-refinery was built at Novopolatsk, the site was presumably chosen for geographical reasons, being close (in Soviet terms) to the Comecon countries which would be provided by pipeline with Soviet oil. Since then, however, oil has been located in the Byelorussian Republic itself, giving a yield in 1976 of some millions of tonnes. Some seventeen oil deposits have been located to date, and new techniques have been developed permitting the exploitation even of those around Mazyr, where the geological structure is not amenable to standard drilling techniques.

The search for oil has also yielded serendipitous results: potassium and rock salts, lignite, mineral resources, building stone and also non-ferrous metals have been discovered, as well

as new sources of drinking and spa waters. This mineral wealth has come to light in an area of Irish-type rural economy where even collectivisation could not break the traditional dependence on potatoes, peat, and cattle-rearing. The development and exploitation of the new resources should provide a fascinating study in years to come.

● In May 1976, *Pravda* proclaimed that the ninth five-year plan (1971-76) had been a period of intense implementation of laser technology in the national economy, stating that every rouble invested in laser R&D has yielded some 4-5 roubles of income. A number of practical applications of lasers were noted, such as the hardening of dies and metal-working instruments and the drilling of jewels for watches; more significant however, was the fact that an actual investment to expenditure ratio was mentioned, a comparative rarity in Soviet reportage which presumably indicates a particularly favourable return.

Since the current five-year plan began, the media have described a number of more exotic uses of lasers. These range from acupuncture (a recognised medical technique in the USSR) to the use of a laser-beam guidance system for the blind landing of aircraft at night or in adverse weather conditions. At the Latvian State University's astronomical observatory, a senior scientific worker, Maris Abele, and a senior engineer, Augusts Rubans, recently received the Silver and Bronze medals respectively of the Exhibition of All-Union National Economic Achievements for developing a laser range-finder for tracking satellites and space-probes.

Laser irradiation has been used to encourage the germination of seeds, and the Central Aerological Industry of the Hydrometeorological Service has produced a special laser reflector which is now being used for tsunami research in the Kuriles. Laser direction finders for determining the direction of mine-galleries are in use and are being exported to other members of the Comecon bloc. Naturally, nothing is said in these enthusiastic reports about possible military applications of the laser, and the outsider is left free to judge whether this emphasis of the peaceful and diverse use of lasers covers a classified programme, or whether, as in the now-notorious case of titanium alloys, the prolific references in the open press are evidence that no breakthrough of military significance has yet occurred.

Vera Rich

BRITAIN

Advice and consent

The UK government last week published a White Paper (Cmd. 6820) in response to the Flowers Commission report on nuclear power and the environment. Chris Sherwell reports

THE bare bones of the UK government's 17-page response to the Flowers report give those involved in Britain's nuclear debate something to chew over, but many will wonder whether it was worth the eight-month wait. The White Paper says the government has decided to accept the bulk of the Flowers Commission's recommendations. The most concrete action proposed concerns management of radioactive waste. The government also plans to broaden the range of advice it receives, and wants more information made available to the public to facilitate full discussion.

It is only at the end of the White Paper and in the context of public discussion that one of the hard foci of Britain's nuclear debate, the demonstration commercial fast breeder reactor (CFR1), actually surfaces. The government accepts, says the White Paper, that before any decision is taken on CFR1, a special procedure for achieving a proper framework for wider public debate should be settled and announced. Presenting the White Paper last week, the Environment Secretary, Mr Peter Shore, confirmed that CFR1 would not proceed until there had been a broad-ranging examination and debate. He cited the machinery of the Public Inquiry Commission which was available but had not been used as one possible course.

In accordance with the Flowers Commission's recommendation responsibility for the management of radioactive waste has now been given to the Environment Secretary together with the heads of the Scottish and Welsh offices. The government has also accepted in principle Flowers' suggestion for a Nuclear Waste Management Advisory Committee, though it thinks that initially this need not be a statutory body. It also sees no need for a decision yet on the suggestion for a Nuclear Waste Disposal Corporation, but says it will reconsider this in light of a review of existing arrangements for the control of waste which the government is now, according to the White Paper, carrying out "as a priority task".

On both waste management and radiation standards the White Paper announces important changes affecting the control of research. The Secretaries

of State for the Environment, Scotland and Wales will "assume control of the waste management element" in the UKAEA's total R&D expenditure. And responsibility for initiating and co-ordinating research into the effects of radioactivity on man and the environment can, says the White Paper, "be more appropriately performed by a Minister than (as the Commission suggested) by the National Radiological Protection Board (NRPB)".

Mr Shore amplified on this reasoning last week only to say both research and policy were in the public domain. Responsibility would again be exercised by the three ministers acting jointly. So will "strategic responsibility" for monitoring environmental pathways lie with the ministers rather than with the NRPB. The government does accept the Commission's recommendation that the NRPB should have a statutory responsibility for advice on the adequacy of radiation standards for workers and the general public. The membership of the NRPB and its sources of finance are now being reviewed.

On nuclear safety generally the advice the government now receives from the Nuclear Installations Inspectorate, which is part of the Health and Safety Executive, will be supplemented by advice from another independent and expert source. This will be the Advisory

Committee on the Safety of Nuclear Installations, which is being set up in place of the former Nuclear Safety Advisory Committee to advise the Health and Safety Commission.

The pieces of this institutional jigsaw will be increased further by the creation ("pretty soon", according to Mr Shore) of what the White Paper calls a high-level independent body "to advise specifically on the interaction between energy policy and the environment". And there is still Mr Benn's Energy Commission to come.

The White Paper emphasises that a decision both on the choice of thermal reactor (which a junior minister at the Energy Department, Dr John Cunningham, last week said was "imminent") and on the further development of the breeder, would not of themselves involve any commitment to a large additional nuclear programme. "Much more significant" decisions will be needed in a few years' time, it says. A Green Paper on energy policy later this year is expected to spell this out in greater detail.

An air of caution is further conveyed early on in the White Paper. International discussions on nuclear policy, it says, mean that the government is "not yet in a position to give a final response" to certain conclusions and recommendation from Flowers on the two "central issues" of waste and security. That more than anything else confirmed that in nuclear power politics, almost nothing is final. □

Lords judgment

EUROPEAN policies are never easily come by, least of all in energy. With the next chance for the Nine to take a step towards a common policy coming on 14 June, when the EEC Council of Energy ministers meets, more worried voices have added a note to the chorus of concern that normally prevails.

It comes from the House of Lords, whose Select Committee on the European Communities is one body in Britain that seeks actually to dissect the stream of proposals and consultative documents emanating from Brussels. Last week the committee released the latest example of its valuable work—its 28th report—to coincide with a debate in the House.

Coming from sub-committee F, which deals with energy, transport and research, the report examines three energy documents prepared by the Commission for the Council of Ministers. One compared EEC objectives set in 1974 with member states' forecasts of their own production and demands; the second warned of the lack of progress towards the aim of greater self-sufficiency; the third focused on the coal industry.

The committee's criticism is almost unsparing. No reliable conclusion can be drawn from the Commission's statistics, it says. There are unexplained inconsistencies and omissions in the figures. Important assumptions are insufficiently discussed, let alone justified. And the figures are not clear and persuasive enough to encourage the Council to take any important decisions, and could even provide an excuse for inaction.

The committee considers that the Community's attention should be concentrated "less on objectives and forecasts and more on the successes and failures of national policies, on the reasons for them, and on research into means of improvement, both technological and social".

One problem may be a surfeit of analysis and of discussion. As Lord Sherfield, a former head of the UK Atomic Energy Authority, commented in debate last week: "While the clock ticks it is difficult to find much that is new to say about the problem until something is actually done about it".

Chris Sherwell

IN BRIEF

OTS delay

Last week it was announced that the launch of the European Space Agency's (ESA) orbital test satellite (OTS), designed for experiments in communications, would not go ahead as scheduled next month because of damage to the American Delta 3914 launch vehicle. In April, ESA's geostationary scientific satellite, Geos, was thrown into a wrong orbit because the Delta 2914 rocket which launched it failed to separate according to plan.

The launch of OTS had already been delayed by the enquiry into the mishap over Geos. The damage to the Delta 3914 occurred when one of the nine solid fuel booster rockets attached to the launcher base fell off, damaging the rocket's first stage. The rescheduled launch date is 8 September; the Meteosat satellite, which was to have been launched on 15 September, has also been rescheduled, for 3 November. ESA decided that it was prefer-

able to delay both satellites by a few months.

OTA director resigns

Emilio Daddario, the first and only director of the Congressional Office of Technology Assessment (OTA), submitted his resignation last week. In a letter to Senator Edward Kennedy, chairman of the joint committee that controls OTA, Daddario noted that the fledgling office has now completed several major studies and he pointed out that he had long expressed a desire to leave as soon as OTA was firmly established. Established four years ago to provide advice to Congress on technical issues, OTA has encountered a difficult problem in giving long term advice to a body whose attention span is largely governed by the two-year election cycle. It has had a mixed reception and last month Kennedy asked the OTA's Independent Advisory Committee to con-

duct a thorough analysis of the office's role and operations. There have been rumours that Kennedy initiated the study and engineered Daddario's resignation as a prelude to taking firm control of OTA. Such rumours were rife when OTA was first established and Kennedy was made chairman of its Congressional board, but they have since proved groundless.

SRC support for AGT

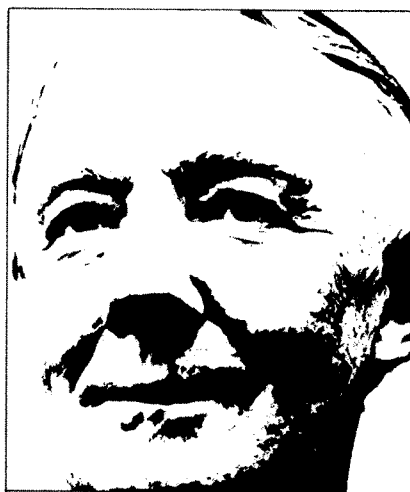
The UK Science Research Council (SRC) is to increase its support for basic research relating to Advanced Ground Transport (AGT). A UK government White Paper released last week says the SRC hopes to spend up to £200,000 a year, concentrated mainly in the universities. This is more than the sum that the SRC has spent altogether over the past three years, but will be less than that spent from all sources over the period 1973-76; this totalled over £1 million.

AN elderly visitor carrying baggage to his bedroom in the usual porterless hotel in modern Britain is likely to suffer at least minor abrasions as he wrestles with the heavily sprung fire doors which obstruct the corridors every few yards. The cost of installing these precautions has meant that hotel charges have risen much more rapidly than inflation alone would have dictated, and many cheaper hotels have closed.

It is right that we should try to make all our activities safe, and new laws may sometimes be required. There have been tragic hotel fires, some buildings are unnecessarily dangerous, but many modifications serve little purpose except to make work for inspectors and builders. The British Health and Safety at Work Act of 1974 contains useful provisions, but its application to laboratories is often governed by dogma rather than commonsense. Precautions, costly in time and money, are taken against the most unlikely catastrophes. Little research has been done to identify the probable dangers in different types of laboratory, where quite different work is done. Our Research Councils must be spending more than £2 million a year, out of their meagre funds, on 'safety'.

This is all part of the 'nanny' to which we are increasingly subjected. We see those who spend their holidays rockclimbing or even in that most dangerous of sports—motorcycling—insisting on following quite different rules when at work. The various admirable and tough outdoor

activities for young people described in his broadcast by the Prince of Wales, and to be supported by the Queen's Jubilee Appeal, will surely

Dangers of safety**KENNETH MELLANBY**

be regulated out of existence.

I find most ludicrous the proposals to set up working parties to draft codes of practice for field work. Field work covers many different activities, some of which may be dangerous, and where obvious and well established precautions should be observed. But for the ecologist it is seldom more hazardous than a gentle country walk to observe birds or to pick wild flowers. Those of us who have done this sort of work should be grateful that someone actually

paid us, for many of our less fortunate friends do these things, possibly less systematically, when on holiday. I expect the code will tell us to change our socks if they get damp, and to wrap up well in cold weather. It is unlikely that any joker will be able to produce 'spoof' regulations which will be more absurd than those to be promulgated by the authorities.

We avoided this nonsense in the past because most field work was done almost entirely by responsible scientists themselves, walking on their own feet and observing with their own eyes. Today there is a growing tendency for scientists to remain indoors in their 'offices' and send assistants, admirable workers no doubt, but not personally committed to the investigation, to do the field work. The results of this practice are often, scientifically, disappointing, and it is understandable that the assistants' trade unions should be obsessional about their safety particularly when this increases opportunities for employment.

However, even the best schemes may have unfortunate results. On what was, by coincidence, the first day of European Farm Safety Week an estate worker drove his tractor a little too quickly over a 'sleeping policeman' (a ramp to slow traffic and prevent accidents). He bounced into the air, and hit his head on the roof of his newly-fixed safety cab, so that he had to be taken to hospital to have his scalp stitched. Too many safety precautions can be jolly dangerous.

news and views

An induced birth for the Solar System?

from M. G. Edmunds

DID the Solar System, like the Universe, begin with a bang? The idea that the supernova explosion of a star initiated the formation of the Solar System has recently been re-explored by A. G. W. Cameron and J. W. Truran (*Icarus* **30**, 447; 1977). The model they discuss may also have interesting consequences in deciding what astrophysical objects liberate significant amounts of elements formed during intense neutron irradiation.

The motivation for their renewed interest is an attempt to account for certain radioactive isotope abundance ratios in the early Solar System, as determined from chemical analyses of meteorites. Particularly important is the unusually high abundance of ^{26}Mg found in aluminium rich minerals in the Allende meteorite by T. Lee, D. A. Papanastassiou and G. J. Wasserburg (*Astrophys. J. Lett.* **211**, L107; 1977). The high magnesium isotope abundance implies that its rapidly-decaying progenitor ^{26}Al had been produced not more than a few million years before the condensation of solid bodies in the Solar System. The extreme temperature and pressure conditions of the supernova explosion of a massive star are the site of synthesis of heavy elements. Cameron and Truran therefore argue, since the unrelated chance occurrence of a supernova just before the Solar System formed is unlikely, that the aluminium synthesising supernova must have induced the formation. A shock wave from the supernova explosion passes through the interstellar medium, and this extra pressure on a cool region of somewhat higher density than average, might be sufficient to drive the region into a state of gravitational collapse. This collapsing cloud would then form the solar nebula out of which the Sun and planets formed. Nuclei in the expanding supernova remnant would mix with the cloud material, and by considering isotope ratios, Truran and Cameron estimate (with a large uncertainty) that perhaps 2% of the heavy elements—anything

from carbon onwards—in the Solar System came from the nucleosynthesis zones of the triggering supernova.

The supernova material could be delivered in either gaseous or solid form. Cameron now accepts that solid grains may well condense out of the synthesised heavy elements as the remnant expands and cools. Thus the radioactive nuclei could be incorporated into grains and directly transported, with their products as they decay, into the Solar System material. Subsequent heating of the grains during or after their incorporation into larger solid bodies may well have caused some chemical differentiation and loss of individuality, so that the supernova grains may not be identifiable with particular inclusions in the meteorite. Lee *et al.* give evidence that the inclusions containing high ^{26}Mg must have crystallised when both freshly synthesised ^{26}Al and older material were present, implying that the synthesis must have occurred only shortly before the solidification of the meteorite. Although the ^{26}Al could have become incorporated into grains, the grains must have been freshly made and then melted during or after meteorite formation. The mineral evidence seems to make it unlikely that the ^{26}Mg could have been brought in by old supernova grains long after its production by decay of ^{26}Al , and then directly incorporated as single grain inclusions. Production of ^{26}Al by processes other than supernova synthesis is unlikely, since those which have been proposed predict other isotopic anomalies which are not observed.

Apart from triggering the collapse of the protosolar cloud, the influence of the supernova on the formation of the planets would also be felt in the effect of decay of ^{26}Al as a heat source. Lee *et al.* suggest that the cores of any solid bodies greater than a few kilometres in radius would be melted while the aluminium was still radioactive.

Having a supernova just before the

Solar System formation is not without its embarrassments, since the model requires a revision of earlier ideas on the use of some other isotope ratios as indicators of the time elapsed between formation and the last nucleosynthetic event which contributed material. For example, it is assumed that ^{127}I and ^{129}I are produced in the r-process, in which nuclei capture neutrons in a flux strong enough to give insufficient time for β -decay of the intermediate product nuclei before the addition of the next neutron. In this process ^{129}I and ^{127}I should be produced in about equal amounts. Now ^{129}I is unstable, and the $^{129}\text{I}/^{127}\text{I}$ ratio at the formation of solid bodies in the Solar System used to be used as an indicator of how long the ^{129}I had been decaying since it was produced in an r-process event. The experimental abundance ratio of 10^{-4} implied that the last ^{129}I was synthesised as long as 10^8 years before solidification, obviously long before the ^{26}Al synthesis. This implies that the event which made the ^{26}Al could have released very little r-process material to the interstellar medium. Truran and Cameron propose an alternative in suggesting that all the ^{129}I observed in the Solar System was synthesised in the ^{26}Al -producing event, but by a process involving slow neutron capture to reduce the production ratio by two orders of magnitude. If 2% of the supernova material were incorporated in the Solar System, this would then give the experimental abundance ratio.

This removes the constraint that the last event which contributed r-process nuclei to the Solar System occurred about 10^8 years before formation. It could then have occurred much longer before, since it is no longer required to synthesise the ^{129}I . A similar argument applies for ^{244}Pu . The attraction claimed by the authors is that the ejection of r-process material from

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supernovae can now be regarded as a very rare event. This is important in considerations of the removal of r-process elements from where they are formed near the core of a supernova, before they get trapped in a condensed neutron star or black hole remnant. The most plausible mechanism invokes perturbation of the supernova structure by strong magnetic fields during the collapse of the core. The difficulty is that magnetohydrodynamic calculations indicate that a very large amount of r-process material must inevitably be liberated, and if this occurred in every supernova then the Galaxy would be inundated with far too many r-process nuclei. The previous argument against this mechanism was that, since by a 'Cosmological Principle' the Earth is supposed to occupy no special

position in the Galaxy, it was unlikely that a rare r-process event occurred not very long before formation. This can be relaxed, since there is now no time constraint on when the last r-process contributing event occurred.

The idea that the Solar System origin was induced by a supernova shock wave is not likely to prove unpalatable, since a similar mechanism of cloud collapse induction is believed to cause the formation of bright massive stars in the spiral arms of the Galaxy as the result of the passage of a propagating density wave in the interstellar medium. Nucleosynthesis and Solar System-formation theorists may be rather more reluctant to throw away their useful, but perhaps misleading, timescales previously derived from some of the isotopic ratios. □

Optimal life-history strategies

from Robert M. May

THE idea that an organism's life-history strategy—its patterns of mortality, fecundity and parental care—depends on its physical environment and on its interactions with other organisms dates

back at least to Darwin and Wallace.

Stimulus for a more quantitative approach to the subject came from the work of people such as Deevey (*Q. Rev. Biol.* 22, 283; 1947) and Cole

(*Q. Rev. Biol.* 29, 103; 1954). On the empirical front, Deevey showed how data from natural populations of animals could be used to construct actuarial tables of age-specific survivorship and fecundity, similar to those constructed by insurance companies for human populations. On the theoretical front, Cole made the remark, which at first glance may appear paradoxical, that a population of immortal organisms, each of which produces N offspring each year, has the same growth rate as a population of organisms of which each lives for one year and then dies after producing $N+1$ offspring. The proof of this result, and its generalisation to somewhat more realistic situations, is trivial (although the simplicity is often concealed in thickets of algebra). So long as the survival probability is independent of age, and all organisms reproduce each year, then adults and juveniles have the same potential for future reproduction and 1 adult plus N offspring is reproductively equivalent to $N+1$ offspring. By extension of this argument, if the probability of a juvenile surviving the first year of life is smaller than the adult survival probability by a factor p , then $1/p$ additional offspring are required if the 'annual' life-history strategy is to have the same population growth rate as the 'perennial' one.

More recent studies have emphasised that the number of offspring, and their chances of surviving to maturity, depend on the amount of parental investment (for example, on the number and size of eggs, and on the amount of care, if any, given to offspring), and that this in turn influences the parent's survival probability. To quote one of a multitude of examples, Loschiavo (*Canad. Ent.* 100, 86; 1968) has demonstrated an almost inversely linear relation between the number of eggs laid and the longevity of the beetle *Trogoderma parvum*. Stearns (*Q. Rev. Biol.* 51, 3; 1976) catalogues other examples. Thus in any attempt to calculate optimum life-history strategies for particular environmental circumstances, it must be acknowledged that age-specific mortality and fecundity schedules are not independent, but are intertwined in a complex way; the trade-off between reproduction and 'personal growth' made by an adult in any one year will affect its probability of surviving that year, and thereby influence the possible trade-offs in all future years.

An excellent review of most work in this area up to 1975 has been given by Stearns. Schaffer (*Ecology* 55, 291;

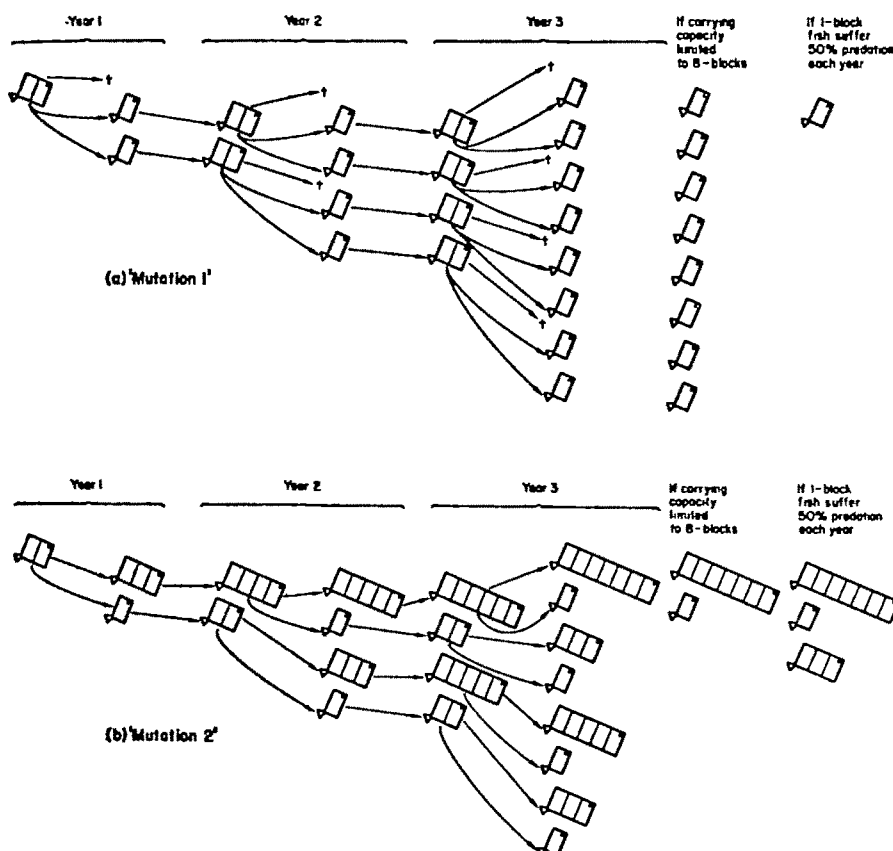


Fig. 1 The outcome of two different strategies for allocating resources in a hypothetical animal, the block-fish. *a*, Mutation 1 in which summer productivity (two blocks) is entirely allocated to reproduction; *b*, Mutation 2 in which one block of summer productivity is allocated to reproduction and one block to growth and, hence, to adult survival. (After Southwood, *op. cit.*)

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1974; *Am. Nat.* **108**, 783; 1974), Goodman (*Am. Nat.* **108**, 247; 1974) and others have given mathematical discussions, and Pianka and Parker (*Am. Nat.* **109**, 453; 1975) have subsequently given a graphical analysis of the way optimal strategies are moulded by trade-offs between present and discounted future reproduction. A discussion which is particularly simple and clear, yet which retains all the biological essentials, is due to Southwood (Ch. 3 in *Theoretical Ecology*, Blackwells (UK) and Saunders (USA), 1976). He invokes an imaginary animal, the parthenogenetic block-fish, which has a productivity of two blocks each summer and one block each winter. The two summer blocks can be added to the fish itself, or used in reproduction, in any proportion (any fish that does not add to itself dies); the one winter block is necessarily added to the fish itself. What is the optimal life-history strategy for the block-fish? Obviously there is no unique answer, but rather it depends on the environmental setting. Figure 1 illustrates the genealogy of two mutant strains of the block-fish: mutation 1 puts all its summer productivity into reproduction; mutation 2 puts only half its summer productivity into reproduction. If half the juvenile one-block fish are killed by predators each year, mutation 2 does better. Conversely, if the carrying capacity of the fish's environment is limited to a total of eight blocks, mutation 1 is represented at the end of three years by many more individuals, and is better placed to "bounce back" from adverse environmental fluctuations. Other assumptions could clearly be explored; for example, block-fish over a certain size could exploit additional resources, or mortality could be higher or lower for larger block-fish, or the environmental carrying capacity could be subject to random variations.

The insights provided by his imaginary block-fish, and by earlier work, enable Southwood to draw together a great deal of information about the trend in life-history strategies manifested by animals and plants, and particularly by birds and by insects. It would be nice to have more experiments aimed at explicit determination of the trade-off between present reproduction *versus* survival and future reproduction. Some such work exists, mainly directed at the relative investment between reproductive and vegetative parts in plants (see for example, Harper & Ogden *J. Ecol.* **58**, 681; 1970; Gadgil & Solbrig *Am. Nat.* **106**, 14; 1972), but by and large it is easier to issue hortatory calls for such experiments than to do them.

Of particular interest is the study of

the optimal reproductive strategy for eusocial insects given by Macevicz and Oster in the new journal *Behavioural Ecology and Sociobiology* (**1**, 265; 1976). They begin by observing that, for colonies of eusocial insects with an annual life cycle, the optimisation problem is to find the allocation of colony resources that maximises the total number of reproductives produced by the end of the colony cycle. For simplicity, Macevicz and Oster assume only two castes, workers *W* and reproductives *Q*. Differential equations are constructed to describe how the growth in the number of workers and of reproductives at any given time depends on *W* and *Q* themselves, on the resources *R* gathered by the workers, and on the 'control variable' that measures the fraction of resources devoted to producing workers, rather than reproductives. A third equation relates *R* to the ergonomics of the colony (the time and energy budgets of foragers); such a relation has been developed and compared with data by Oster (*Am. Nat.* **110**, 215; 1976), but Macevicz and Oster are content to use a simple phenomenological equation. Standard techniques from optimal control theory are then used to find the strategy that maximises *Q* at the end of the cycle. Although presented lucidly and unpretentiously, all this is an order of magnitude more sophisti-

cated than previous work on optimal life-history strategies.

The solution, which emerges independent of many of the details, is a so-called 'bang-bang' control policy. In the first phase, the total reproductive effort is directed towards producing workers. Then, at a critical time t_s approximately one generation time before the season ends, the entire effort is thrown into producing reproductives (queens and males). The theoretical optimal control solutions are confronted with data on the wasp *Vespa orientalis* and on the hornet *Polistes fuscatus*. In both cases, the discontinuous 'bang-bang' policy is evident, and theoretical and actual values of t_s agree to within about 1 day. For *V. orientalis*, the observed and predicted number of workers and reproductives are in remarkable agreement over the entire colony cycle; for *P. fuscatus* the agreement is only fair.

Macevicz and Oster point out that discontinuous 'bang-bang' control policies will characterise a wide class of related optimisation problems, and, in particular, that such policies necessarily apply to any colony or population that undergoes exponential growth throughout its cycle. Realistic complications, such as non-linear density-dependent effects or time delays, may or may not lead to smoothly varying control. □

Superfluid helium: untying the tangle

from P. V. E. McClintock

THE celebrated superfluid properties of helium-4 only exhibit themselves while the liquid is being treated fairly gently. Up to a certain critical velocity it can flow through a tube in a completely frictionless manner, without needing to be pushed to keep it moving. At higher speeds, however, this simple superfluid behaviour disappears abruptly as turbulence is generated and a corresponding flow resistance appears. The resultant turbulent state of the superfluid is of considerable intrinsic interest, particularly in view of the quantum properties of the liquid. A recent paper in *Physical Review Letters* (**38**, 551; 1977) by K. W. Schwarz of the IBM Research Centre at Yorktown Heights represents the first serious attempt to tackle the dynamics of the turbulence and, judging by comparisons with experimental data, his theory seems to be remarkably successful.

Surprisingly, turbulence in superfluid helium is very much simpler than in other liquids, mainly because the so-called quantisation of circulation means that the turbulence takes the

form of a mass of vortex lines such that any element of vortex line is identical to any other. Each vortex line consists of a narrow core region, which may be imagined as being hollow, surrounded by superfluid which circulates rapidly close to the core and more slowly at large distances from it. Turbulence consists, then, of a seemingly random tangle of these vortex lines in constant relative motion because each element of line exists in the flow fields around all the other lines. The situation has been likened to a mass of moving spaghetti, or a basket of writhing snakes (although free ends would be unstable, so one is really obliged to imagine spaghetti in tangled rings, or snakes which have swallowed their tails). These analogies must be treated with caution, however, because in reality the spacing between neighbouring segments of line is very much greater than would seem to be implied.

Most of our present understanding

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of the turbulent state is due to the work of W. F. Vinen (*Proc. R. Soc. A* **240**, 114, 128, 1957; *A* **242**, 493, 1957; *A* **243**, 400, 1958). He envisaged that, in the equilibrium situation, the rate at which new vortex line was being created by 'frictional' effects would be exactly balanced by an annihilation mechanism whereby vortex line would be being continually destroyed. His theory, which was based largely on dimensional considerations, had nothing to say about the details of either the creation or the annihilation mechanisms, so that there has been a continuing debate about what might actually be going on in the liquid. One school of thought held that the lines were annihilated when, in the course of their random movement, they crossed each other, while others believed that such a crossing would be able to occur without the lines even 'noticing' each other, let alone being an event which would lead to their mutual destruction. Sitton and Moss (*Phys. Rev. Lett.* **29**, 542; 1972) suggested that the annihilation mechanism arose from the magnus force which tends to drive together parallel sections of line with opposite circulation; but this idea was not supported by subsequent experimental work by Ashton and Northby (*Phys. Rev. Lett.* **30**, 1119; 1973). The situation has remained obscure.

There have, in fact, been growing indications of the inadequacy of the Vinen model, arising mainly through experiments in which charged ions are used to probe the movements of the tangle of vortex lines. In one such experiment, Ashton and Northby (*Phys. Rev. Lett.* **35**, 1714; 1975) were able to demonstrate that the tangle was not moving at the same speed as the superfluid (in fact, it was travelling in the opposite direction), thus contradicting one of the central assumptions of Vinen's theory, that the tangle of lines must be isotropic. It was clear, therefore, that a more sophisticated approach was needed.

What Schwarz has now attempted is to take some explicit account of the actual way in which the lines move relative to each other and to distant parts of themselves: a daunting task which has, of course, required a statistical approach. His central equation, describing the rate of change in the length of line per unit volume of liquid, is extremely complicated, but contains no adjustable parameters. An interesting feature is that it seems to imply, quite contrary to the premises of earlier theories, that the most important velocity is not that of the random inter-line movements, but is that of a segment of a line relative to other segments of the same line. The solution

of this equation has necessitated the use of numerical methods. It turns out that, for any given temperature and flow velocity of the liquid, a forward integration in time leads to the same steady-state distribution of vortex line, regardless of the assumed initial distribution of lines: apparent confirmation that the theory is at least self-consistent. More importantly, it seems to be in remarkably good agreement with experiments. Contrary to the Vinen theory, it predicts that there will indeed be a relative velocity between the vortex tangle and the superfluid, with the tangle tending to move in the same direction as the thermal excitations in the liquid. Furthermore, both the magnitude of this velocity and its temperature dependence are in good agreement with the measurements of Ashton and Northby. The predicted line density turns out to be in satisfactory agreement with Vinen's original measurements; and, again, the calculated temperature dependence is as it should be.

These results are extremely encouraging. It will be interesting to see whether the model will be able to deal equally successfully with critical velocities for the onset of turbulence, and with the transient situations which occur when turbulence is building up or dying away in the superfluid. No doubt there will be a brisk renewal of interest by experimenters, now that there is a seemingly satisfactory theory waiting to be verified or contradicted.

What can artificial intelligence offer psychology?

from Tim O'Shea

A meeting entitled 'What can artificial intelligence offer to psychology' was held at the University of Bristol on 14-15 April, 1977. It was organised by Richard Young (University of Edinburgh) and Tony Conway (University of Bristol) for the Society for the Study of Artificial Intelligence and Simulation of Behaviour (AISB).

ARTIFICIAL Intelligence is an investigation into the principles underlying actual and possible forms of intelligent behaviour. In part this objective is shared with psychology which is, however, concerned with human and animal behaviour. The methodology of artificial intelligence, unlike that of psychology, is based not on experimentation with people and animals, but

on the design, implementation and analysis of computer programs which exhibit intelligent behaviour. The aim of the meeting was to examine the relevance and applicability of this methodology to psychological research. Most of the speakers were active research workers in artificial intelligence but many of the other participants were cognitive psychologists. The work presented ranged in application from H. C. Longuet-Higgins' (University of Sussex) program for the perception of melodies to S. Weir's (University of Edinburgh) account of therapeutic work carried out with an autistic child.

Opening the meeting R. L. Gregory (University of Bristol) used the variety of types of intelligent behaviour as the basis for criticising attempts to measure intelligence by a single numerical IQ score. He proposed a distinction between 'design intelligence' (a pre-existing or 'wired-in' intelligent solution to a problem) and 'processing intelligence' (the generation *de novo* of intelligent solutions) and argued that artificial intelligence has made explicit the large amount of 'processing intelligence' necessary for perception and simple skills. He also suggested that the construction of robots with restricted 'design intelligence' provides a way of examining the effective use of limited data.

A large proportion of the talks described fully or partially implemented computer programs. These papers were probably the most useful, as the research methods of artificial intelligence and the current state of the art were made explicit through the discussion of the actual programs. The thesis that artificial intelligence should be regarded as theoretical psychology was cogently put by Longuet-Higgins. He argued that artificial intelligence provides psychologists with an appropriate descriptive medium for the expression of theories. These take the form of computational mechanisms which can be used to specify the complex interacting processes necessary for intelligence. This view of artificial intelligence as theoretical psychology was strongly supported by R. Schank (Yale University) in his discussion of language comprehension. Schank argued that process models are essential in any account of linguistic phenomena and that such models could best be realised as computer programs. He illustrated his argument with an account of a computer program which takes as input stories set in restaurants. It uses 'scripts' of the expected sequences of

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actions in restaurants to 'understand' such a story. The extent of its understanding is demonstrated by its ability to answer English questions about the story, including making inferences about events that must have occurred between the incidents specifically mentioned.

Many speakers argued that the construction of computer models which exhibit intelligent behaviour requires the use of knowledge from a variety of different domains. For example, phonetics, syntax, semantics and pragmatics are all necessary for the synthesis of intelligible speech. This point was nicely illustrated by S. Isard (University of Sussex). He is working on the problem of determining the 'right' intonation contour for a given English sentence taking into account the intended meaning and the context in which it is spoken. Consider the sentence 'I bought a boat and *that* sank'. A different intonation pattern is applied in the case where the speaker assumes it is known that his first boat blew up, from the pattern applied where the speaker is trying to correct the mistaken impression that it was his first boat that sank. Isard described a program which he is developing to explain how the differing contexts in which a sentence is uttered give rise to different intonation patterns. The program produces spoken English generated by a speech synthesiser. (The tape-recordings of the generated speech he played had an oddly Scandinavian lilt.) One argument for the development and specification of psychological theories as computer programs was that they can then be run on a computer and their consequences seen in a clear form. Isard illustrated this by describing various experimental changes he had made to the components of his program and discussing the effects these changes had had on the intonation of the generated speech. He concluded his account by observing with disarming equanimity that 'parts of my program are starting to interact in ways that are making me uncomfortable'.

How best to organise such complex programs remains an open question. A. Sloman (University of Sussex) argued that there was a need to deploy the different components of an intelligent program concurrently rather than sequentially. Sloman illustrated his argument by a description of the interactions between the different levels of a program which interprets visual scenes composed of overlapping letters. Such arguments for the need for parallel processing in computer models of intelligent behaviour were responded to with enthusiasm by some of the psychologists present. But on the whole the psychologists were sceptical of compu-

tational analyses of cognitive functions which were not based on any concrete computer program. The main criticism ironically enough was one often levelled at psychological theories, namely a lack of rigour together with a resulting absence of predictive power. P. J. Hayes (University of Essex) argued that the difference between theories in artificial intelligence and those in psychology is that the former must be implemented as computer programs to be viable, while the latter must make experimentally testable predictions. J. M. Brady and R. Bornat (University of Essex) asserted that the main strength of computer programs as models of cognitive processes was that they were precise enough to be refuted. They contrasted such programs with contemporary models in cognitive psychology which they suggested are couched in computational terms but are not, in practice, implementable as computer programs. D. A. Allport (University of Reading) gave examples of general organisational principles such as heterarchy and debugging which might be applicable in psychological explanations. However he argued that the main benefit of artificial intelligence to psychology so far lies in the carefully detailed analysis motivated by the demands of developing computational descriptions rather than in the application of general principles. He criticised workers in artificial intelligence for paying insufficient attention to known psychological phenomena. While this criticism certainly applies to some of the work presented it could not, for example, be applied to G. Kiss's (University of Warwick) analysis of the computational complexity of some simple cognitive processing tasks. This analysis was directly applied to experimental findings on memory span and other results. Nor could this criticism be applied to the style of development espoused by G. Luger (University of Edinburgh) for a computer program which solves 'A' level mechanics problems. Luger repeatedly emphasised the necessity for analysing protocols of human subjects as a source of design ideas for programs intended to have natural language understanding and problem solving capabilities. Allport also pointed out that much artificial intelligence work is judged against the intuitive sufficiency of the mechanisms used. The validity of this objection was made clear by the ambiguous and easy response of many of the speakers when questioned on their views of computer programs which performed some task requiring intelligence but did not make the same errors as humans.

Despite the gladiatorial format of the meeting there was a surprising degree of consensus between the psy-

chologists and the workers in artificial intelligence. This was particularly apparent during M. Eisenstadt's (Open University) account of a new course in cognitive psychology. This course is based on the artificial intelligence view that representation and symbolic processing are central to the specification of cognitive functions. No one seriously questioned that artificial intelligence is in principle useful to psychology and there was general agreement that there is a large gap between current experimental work in psychology and the construction of computer programs. The difficulty of making empirically testable predictions of human performance (from the execution of computer programs) was repeatedly stressed. In the long term the most valuable contribution of artificial intelligence to psychology may be to liberate it from a methodology based on a quest for simple explanations relating quantitative variables. This search for simple mechanisms underlying human behaviour is characterised by the collection of a mass of experimental data in the absence of precise theories about the underlying cognitive functions. Artificial Intelligence offers a methodology for the development and elaboration of symbolic representations for the processes underlying intelligent behaviour. □

Probing satellite DNA

from Gabriel Dover

THE point has been reached in the study of highly repetitious (satellite) DNA of higher organisms where earlier plausible generalities can no longer significantly advance our understanding of the origin and function of this species of DNA. These generalities were usually based on correlations between aspects of chromosome behaviour (often during meiosis) and the composition, amount and chromosomal location of heterochromatin (or satellite DNA).

Early studies showed that the majority of satellite DNAs are highly homogeneous in sequence and some can differ from each other (whether in the same or different species) by relatively few base changes despite the lapse of long periods of evolutionary time. They are concentrated in sections of the chromosome adjacent to the centromeres and telomeres which are involved in chromosome positioning

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during the cell division cycle. Furthermore there is no concomitant replication of satellite DNA in specialised cells having polytene chromosomes.

These almost diagnostic features of satellite DNA have led to much current interest in the molecular mechanisms responsible for the generation and maintenance of homogeneity of sequence and in its biological role: a situation analogous to the conserved and non-conserved sequences, within and between species, of multigene families and their spacers.

Some answers to these questions can be gained from studies that probe more extensively the sequence organisation of satellites, the finer patterns of satellite distribution on the chromosomes, and the extent of satellite sequence homologies in species of known phylogenetic relatedness.

Important new data obtained by a combination of these approaches is reported on the satellite DNAs of *Drosophila melanogaster* (Brutlag *et al. J. molec. Biol.* **112**, 31; 1977; Shen & Hearst *J. molec. Biol.* **112**, 495; 1977). There are four major satellites in this species (Peacock *et al. Cold Spring Harbor Symp. quant. Biol.* **38**, 405; 1973; Endow *et al. J. molec. Biol.* **96**, 665; 1975) ranging from 2.6–5.3% of total DNA (equivalent to 4–8 million bases each) that are located at the chromocentres of salivary gland cells. If satellites are the basis for the differences in behaviour of pairs of homologous chromosomes it might not be unexpected to find a pattern of distribution of these four satellites that is chromosome specific, and in addition to locate sequence variants of individual satellites on individual chromosomes. Brutlag *et al.* after determination of the composition and properties of major and minor satellite peaks (resolved in antibiotic-CsCl gradients), have demonstrated that there is an upper limit of between 8–10 chromosomal sites for each block of satellite (of average size 750,000 bases) and that these blocks are predominantly interspersed with each other. Their argument is based on the interpretation that the minor peaks represent linkage molecules at the boundaries of contiguously covalently linked blocks and have buoyant densities commensurate with a dual satellite composition. Hybridisation of homogeneous pure satellites to a minor peak associated with satellite IV ($\rho = 1.705 \text{ g cm}^{-3}$) shows that it contains sequences of three flanking satellites (1.705, 1.686 and 1.672), in conjunction with some main-band DNA.

Although there remain several, as yet, untested minor satellites, this interpretation is secure when considered in conjunction with *in situ* hybridisation experiments at CSIRO (Peacock *et al.*,

in preparation), that have revealed chromosome-specific patterns of interspersed blocks of satellite making up the heterochromatin around mitotic centromeres.

The existence of sequence variants within a satellite can be demonstrated with the use of restriction endonucleases as probes to examine the range and types of long periodicities that may overlay the basic short repeating unit. If sites, susceptible to attack by a particular endonuclease, are regularly spaced throughout a block of satellite DNA then the DNA fragments produced after enzyme digestion will be of a uniform size indicative of this high-order spacing. Satellite IV has hitherto proved particularly resistant to a number of restriction enzymes and it has not been possible to detect higher-order periodicities of the sort reported in satellites of other organisms (Southern *J. molec. Biol.* **94**, 51, 1975; Botchan *Nature* **251**, 288; 1974) and in satellite III of *D. melanogaster* (Manteuil *et al. Cell* **5**, 413; 1975). However Endow (in the press) has recently succeeded in cutting it with *Mbo*II, confirming its repeating pentamer purine sequence of (AGAAG)_n and its high (95%) conservation of sequence.

The novel use of 4,5',8-trimethylpsoralen (trioxsalen) on satellite IV by Shen and Hearst in conjunction with denaturation microscopy has revealed a regular distribution of cross-linkable pyrimidine sites at intervals of approximately 250 base pairs and brings this satellite firmly into line with those of mouse, guinea-pig, calf and green monkey in its organisation. This is not only an important step forward in the application of a new method for probing satellite sequence organisation, but also raises intriguing questions concerning the universality of higher-order repeats, the mechanism of their evolution and maintenance and, naturally, their biological role. Recent data by Zachau and his colleagues at Munich on the organisation of mouse, guinea-pig and African green monkey satellite DNAs (Horz & Zachau *Eur. J. Biochem.* **73**, 383; 1977; Altenburger *et al. Eur. J. Biochem.* **73**, 393; 1977; Fittler *Eur. J. Biochem.* **74**, 343; 1977) indicates that satellite evolution is even more complex than at first realised. The pattern of fragment sizes produced after successive- and co-digestions of satellite DNA with several restriction enzymes revealed the existence of satellite sequences (segments) within the block of satellite DNA that are variants of a common ancestral sequence. This is with respect to the organisation of higher periodicity, in that sites sensitive to different restriction enzymes are clustered in different parts of the satellite block.

The stage is now set to investigate the possibility of equivalent segments (sequence variants) in the satellites of *Drosophila* and to relate this to the new data of Brutlag *et al.* and Shen *et al.* Sequence variants of a satellite could reside either on different homologous chromosomes (thus reinforcing the chromosome differences in the patterns of satellite distribution) or in other species that are closely related to *melanogaster* and that have some satellites in common. Such comparisons, currently underway by Sederoff and his coworkers in Oregon; at Canberra (Australia) by Peacock's group; and at Cambridge, UK on the *melanogaster* species group, might reveal something of the order of events in the evolution of satellite DNAs that has led to their contemporary composition and distribution both within and between closely related genomes and resolve some of the intriguing problems associated with this enigmatic species of DNA. □



A hundred years ago

"SUMMER Schools" are becoming a regular institution in America, and no more pleasant way could be devised of combining the *dulce* and the *utile* than that of a proposed aquatic summer school of natural history, which, under the direction of Prof. Theodore B. Comstock, of Cornell University, expects to charter a large steamer, and spend the summer around the shores of Lake Superior in the study of the geology and natural history of that region. The steamer will accommodate from seventy-five to one hundred passengers, to be made up of students and professors. Regular instruction will be given in the form of lectures during the voyage, and every facility afforded by the examination of mining localities and the like. The vessel will probably start from Cleveland or Detroit on July 7, and proceed thence to Lake Superior, making its full circumnavigation. The coast of Georgian Bay, on Lake Huron, will be investigated on the return voyage. The expense will probably amount to about 125 dollars for each person. In addition to the aquatic summer school mentioned above, Harvard University announces two special summer courses of instruction, one in zoology and the other in geology.

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articles

History of the Mediterranean salinity crisis

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A history of geodynamic evolution of the Mediterranean leading to the salinity crisis is outlined, based on the 'desiccated deep-basin model'. An accurate portrayal of the crisis is presented, based on data from new drilling and studies of on-land geology.

THE discovery of the Mediterranean Evaporite by the drilling from Glomar Challenger in 1970 proved that salt exists under deep-sea floor¹, and demonstrated that giant salt deposits could have been formed within a relatively short geological time². The almost synchronous onset and termination of the Mediterranean salinity crisis implies catastrophic changes of environments in a region over two and half million square kilometers in extent. This fact did much to throw doubts on Lyell's substantive uniformitarianism³.

The significance of the Deep Sea Drilling Project (DSDP) discovery was, however, shadowed by controversies. That the Mediterranean Evaporite is Messinian (Late Miocene) in age seemed to be just about the only consensus after the Leg 13 drilling. Even the shipboard scientific staff could not reach an agreement on the genesis of this unusual formation. Chapter 43 of the Leg 13 cruise report presenting the desiccated deep-basin model (ref. 4) was authored by K. J. Hsü, Maria Cita, and W. F. B. Ryan because they were the only members of the shipboard staff totally convinced of its plausibility. Alternative interpretations were given by other shipboard staff in the Initial Cruise Reports and later in other publications^{5,6}. The reactions of the scientific community to the desiccated deep-basin model were also divergent. Favourable commentaries were not rare. On the other hand, many critiques were sceptical, or downright hostile.

The importance of clarifying the question of the Messinian salinity crisis did not escape the attention of the JOIDES organisations. A second cruise to the Mediterranean took place in 1975. We are glad to report that we reached a near-consensus on the previously controversial question of the origin of the Mediterranean Evaporite. This article is not a perfunctory report requiring a co-authorship by the shipboard scientific staff. It is a

unanimous opinion written by the senior author for ten co-authors, who represent all but two of the shipboard scientific staff during the Leg 42A cruise.

The Miocene Mediterranean

Whereas there seemed to be general agreement that the Messinian (Late Miocene) evaporites were deposited in shallow water conditions, controversy revolved around the basin depths at times of evaporite deposition⁷. The crux of the matter is the genesis of the Mediterranean basins. When did the Mediterranean basins form? In the Early and Middle Miocene before the Messinian salinity crisis⁸? In the Pliocene shortly after the salinity crisis⁹? Or "did the main phase of subsidence occur rather abruptly during the Pleistocene and Holocene"¹⁰. Syntheses of regional tectonic data led to the conclusion that the deep basins of the Mediterranean have evolved to a stage quite similar to their present configuration before the Late Miocene when the salinity crisis began^{4,11-14}. Of the five major Mediterranean basins, the Levantine and Ionian basins may be relic Tethys of Mesozoic age; The Balearic and Tyrrhenian were formed during the Early and Middle Miocene, after the culmination of the Alpine orogeny. Perhaps only the Aegean has undergone significant Plio-Quaternary subsidence.

Deep-sea drilling during the Leg 42A has unearthed convincing evidence that deep Mediterranean basins were in existence before the Messinian. Drilling at Site 372 (Fig. 1) indicated that the Balearic Basin was formed by rifting during the latest Oligocene or earliest Miocene time. Palaeobathymetric analyses on the basis of benthonic foraminifera estimated that this part of the Balearic margin was at least 900 m deep during the Early Miocene (Burdigalian) time. The water depth reached in excess of 1,200 m during the late Burdigalian and subsided gradually to a depth more than 1,500 m before the Middle Miocene ended¹⁵.

The pre-Messinian sequence at Site 372 is mainly hemipelagic, similar to the sediments deposited there today. The planktic and benthic faunas and floras are fully open marine. Aside from benthic foraminifera, psychrospheric ostracods also occur in some Early and Middle

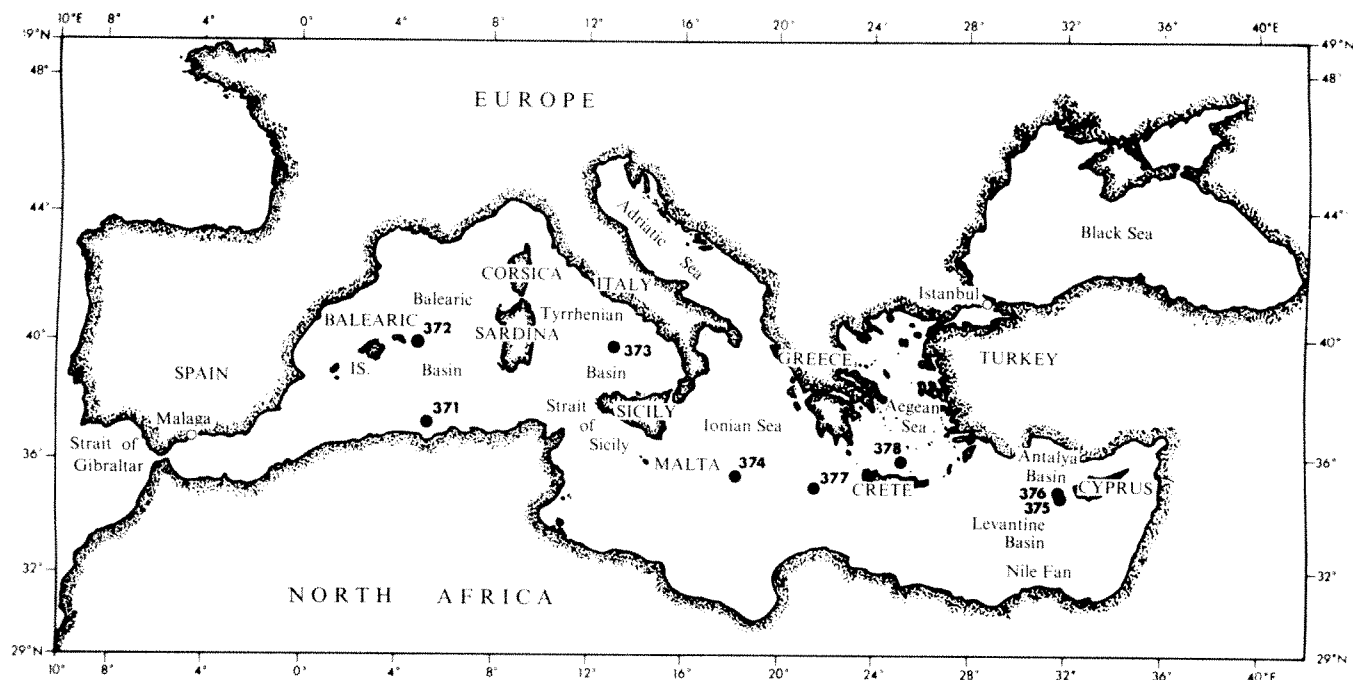


Fig. 1 Drill sites of DSDP Leg 42A.

Miocene cores, indicating water-depths in excess of 1000 m (ref. 16).

Drilling results at Site 373 seamount indicated that a deep Tyrrhenian Basin was also in existence during the Messinian. Palaeotemperature estimated for the submarine cementation (by calcite) of the early Pliocene pillow breccias at this site is estimated by isotopic evidence to be 6–8 °C (ref. 17). Such a low temperature implies that the volcanic seamount was deeply submerged then, only a short time after the salinity crisis.

We penetrated beyond the Messinian at Site 375 west of Cyprus in the Levantine Basin. As in Hole 372, the pre-Messinian sequence at this eastern Mediterranean site is also mainly hemipelagic. Palaeobathymetric analyses indicate that the Levantine Sea was a deep, open sea during the Miocene before the Messinian salinity crisis¹⁵.

Events leading to the salinity crisis

Geological reconstructions indicate the presence of an equatorial ocean, which separated Europe from Africa during the Jurassic and Cretaceous and the ocean was named Tethys^{14,18}. The connection between the Atlantic and the Indo-Pacific was maintained through a shallow seaway after the joining of the two continents in late Cretaceous¹⁹. This shelf sea started to disappear during the Burdigalian (late Early Miocene). This was one of the major palaeobiogeographical events of the Tertiary; it was during this period that "the present pattern of land and sea was established"²⁰. The joining of Europe and Asia permitted the exchange of land fauna between those continents, and started the differentiation of the Indo-Pacific and Atlantic-Mediterranean marine faunas. Whereas the evidence from vertebrate palaeontology indicates the establishment of first intercontinental connection between Eurasia and Africa during the Burdigalian²¹, the micro-palaeontological data suggest that there might have been repeated transgression into the Mediterranean from the Indo-Pacific till Middle Miocene time²² (Fig. 2).

The orogenic movements which fused Eurasia and Africa also raised new mountains in the Taurides, the Hellenides, the Dinarides, and eventually, somewhat later in the Middle or early Late Miocene, the Helvetic Alps. This series of

movements led to a separation of the Mediterranean and an eastern European inland sea, the Paratethys. Stratigraphical and palaeobiogeographical data indicate that the Burdigalian connection between the two seas went through the Peri-alpine Depression north of the Alps^{22,23}. This seaway was eliminated early in Middle Miocene, when the Alps rose and marine waters withdrew from the Molasse trough. A last thread of contact was probably maintained between the Mediterranean (Langhian) and the Paratethys (early Badenian) through an opening in northern Italy and north-western Yugoslavia. The complete separation of the two inland seas took place some 14 to 15 million years ago during the Serravallian (late Middle Miocene). The last Indo-Pacific flooding that invaded the Paratethys later in the Serravallian (14–13 Myr) did not reach the Mediterranean²².

The Middle Miocene separation had two consequences. The Paratethys could no longer receive marine water from the Mediterranean nor could the Mediterranean receive freshwaters from that part of Eurasian rivers which now emptied themselves into the Paratethys. When the connection was open, surplus ions resulted from evaporative excess in the Mediterranean may have found their way to the Paratethys, and were deposited as the lower Middle Miocene salts of the Pannonian and Transylvanian Basins²². After the loss of communication the Mediterranean was deprived not only of much freshwater influx but also of one of its means of the elimination of evaporative waste.

The Mediterranean's last links to the world ocean were the Betic and the Rif Straits. The deep Betic Strait shoaled after the end of the Middle Miocene; no Late Miocene psychrospheric ostracodes were found inside the Mediterranean¹⁶. Yet open marine conditions prevailed in the Mediterranean during the earlier parts of the Late Miocene (Tortonian and early Messinian)²⁴.

The Messinian change from normal marine to an evaporite-forming condition was a sudden event. The palaeobotanical record gives no evidence of an abrupt change in climate. There had been a general trend towards a drier and cooler climate since the Burdigalian, when the Mediterranean was first cut off from the Indian Ocean²⁵. Thus we cannot interpret the change from normal marine to evaporative conditions as a response to climatic factors; the

change had to be related to a final closure of the openings at the west during the latest Miocene. With the isolation and a serious hydrographic deficit, the salinity crisis of the Mediterranean finally became inescapable.

The Messinian salinity crisis

Seismic records suggested a twofold division of the Mediterranean Evaporite²⁶. (1) An Upper Evaporite sequence, with numerous reflectors, up to several hundred metres thick, and consisting of dolomite, gypsum, anhydrite, and some salts. (2) The Main Salt and Lower Evaporite sequence, seismically homogenous salt, up to a thousand metres thick or more, underlain by several evaporite reflectors. The distribution of the Main Salt unit is restricted to the more central part of each of the Mediterranean basins. The distribution of the Upper Evaporite unit is, however, more extensive.

Since drill cores only yielded information on the Upper Evaporite, we depend on land sections for an overview of the Messinian salinity crisis. The onset of the salinity crisis as in Sicily, for example, indicated by the contact of *Calcare di base* on 'Tripoli', signifies a very rapid change from a bathyal environment of hemipelagic sedimentation to a shallow, or subaerial environment of carbonate deposition and diagenesis²⁴.

The history of the early evaporite deposition can be deciphered from a reference to the section of the Cattolica basin, Sicily²⁷. The Lower Evaporite sequence there lies above the Tripoli Formation and includes (1) *Calcare di base*, (2) Cattolica Gypsum, (3) Clastic Gypsum beds, (4) halite and potash salts.

The salts are in general 300–500 m thick. Decima²⁸ divided a 330-m section of salts at Porto Empedocle into four units. The Unit A is a halite with laminae of anhydrite and is 108 m thick. The bromine content of the halite is about 400 p.p.m. The Unit B is well laminated halite, kainite, polyhalite and anhydrite, and is 115 m thick. The bromine content ranges from 200 to 250 p.p.m. except for the uppermost pure halite which contains some 100 p.p.m. Br. The Units C and D overlie the lower salts unconformably. They are 107 m thick and consist of halite, with anhydrite and anhydritic shales. The halite in these upper units has only traces of bromine (10 p.p.m. or less).

The lower salts with their high Br were precipitated from brines derived from evaporated seawater. In contrast the upper salts were recycled. Sedimentological studies²⁹ indicated that the upper salts were deposited in shallow saline environments. On the other hand, the well laminated Unit B, with sequences of cyclic lamination that can be correlated for long distances, were precipitated in a relatively deep subaqueous environment.

The Lower Evaporite is overlain unconformably by a Upper Evaporite of gypsum and marls. A comparison of the submarine Messinian with the Sicily section suggests a similar two-stage evolution of the Messinian salinity crisis in other Mediterranean basins. After an initial desiccation or partial desiccation, the Lower Evaporite was probably precipitated when the supply of brines was sufficiently continuous, to cause the accumulation of thick halite and potash salt deposits on the deepest floor of the Mediterranean basins. Toward the end of the Main Halite deposition, the supply of brine probably ceased because of a complete isolation from the Atlantic. The brine level was drastically drawn down because of evaporative loss. Large areas on the periphery of the salt basins were exposed. Waters from the continent or from local precipitation formed salt creeks, eroding primary salts (for example Units A and B, Porto Empedocle), and depositing secondary salts (for example Units C and D) in the centre of the salt basins. This desiccation period was represented by the widespread unconformity separating the Lower and

the Upper Evaporite units. Rivers cut canyons on the margins of the continents and some of these canyons were eventually flooded by the Upper Evaporite deposits²⁶.

The erosional event was ended by a new inundation of the Mediterranean, when the Mediterranean was probably again filled to the brim. The lower marine marls of Messinian age at DSDP Site 372 (Core 9/2) are the first sediments above the unconformity and they may have been deposited during this intra-Messinian flooding. The subsequent history of the Upper Evaporite sedimentation was probably characterised by periodic influx of marine waters into a largely desiccated deep basin³⁰. A repetition of marine inundation, followed by isolation, and evaporative draw-down led to the accumulation of marls, stromatolitic carbonates, gypsum, anhydrite and salts, in cyclic sequences.

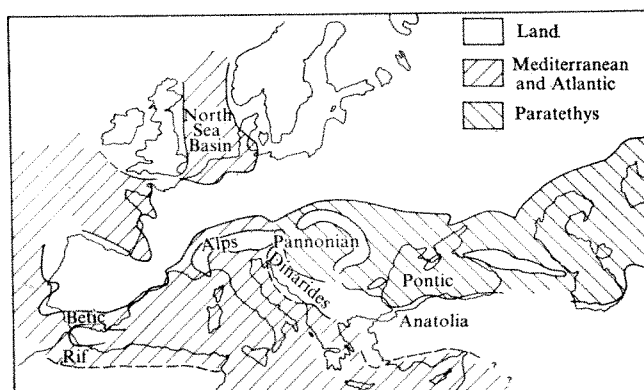
Lago Mare

The geological record of the eastern Mediterranean holes revealed a drastic change in depositional environment during the latest Messinian time before the final Pliocene marine inundation. After the last salt deposition of the Upper Evaporite, the Ionian and Antalya basins were almost totally dried up. Slight changes in the water-budget led to conditions of cyclic sedimentation. The very last Messinian sediments at Sites 374 and 376 are marls, however, deposited in a standing body of water. Isotopic evidence suggests a considerable influx of continental waters to those previously desiccated basins³¹.

Site 376 samples contain an *Ammonia-Cyprideis* fauna, indicative of the euryhaline environment. Similar *Cyprideis* and other euryhaline faunas are present in numerous uppermost Messinian sections on land in both the eastern and western Mediterranean countries. The Mediterranean hosted a series of fresh or brackish water lakes toward the end of the Messinian. We propose that the term *Lago Mare* be used to designate the latest Messinian lake (or oligohaline) environments in the Mediterranean, subsequent to the evaporite-deposition, but before the Pliocene marine sedimentation²⁴.

The change from salt-precipitation in shallow salinas to deposition of marls in *Lago Mare* occurred within a very short time interval probably in less than 100,000 yr. The change implies a radical alteration of the hydrographic budget. There was, however, no drastic climatic change during the Messinian. The environmental change must have been related to a reorganisation of the drainage system in Europe. The sudden appearance of so much fresh or brackish water, together with a typical Paratethyan fauna, probably indicates a sudden inundation of the desiccated Mediterranean by Paratethyan waters from eastern Europe (Fig. 3).

Fig. 2 Middle Miocene (16–18 Myr) Palaeogeography.



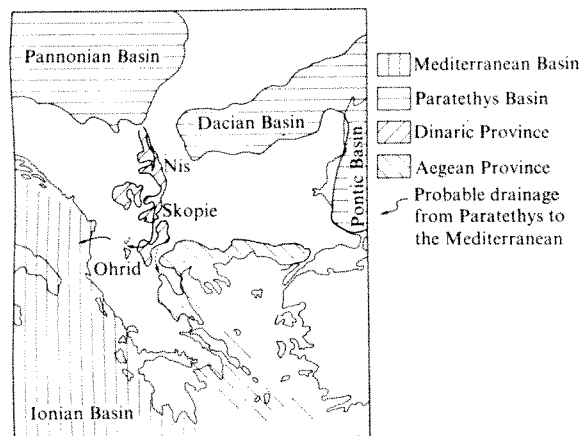


Fig. 3 Late Miocene (5.5 Myr) Palaeogeography.

That such an invasion did take place has been speculated by biogeographers on the basis of the occurrence of relic Paratethys faunas (including freshwater fish, molluscs and birds) in isolated areas of circum-Mediterranean land today³². The evidence from Paratethys also indicated that it suddenly lost much of its waters to the Mediterranean in late Neogene³³. In fact, the drainage reorganisation at this time is believed to have resulted in an evaporative excess of the hydrographic budget of the Black Sea, leading to a partial desiccation of that deep Paratethyan basin³⁴.

The *Lago Mare* may have occasionally extended to the western Mediterranean. The *Cyprideis* fauna is present in land sections in Algeria, Spain, Tuscany, and in Sicily³⁴. A brackish water sediment is present in the Balearic Hole 124 within the evaporite sequence and may be equivalent to the lower part of the *Lago Mare* sequence of the eastern Mediterranean. The very last Messinian sediments there below the Pliocene are, however, restricted marine deposits. It seems that towards the end of the Messinian the marine waters spilled over the western basins before they reached the east. The restricted marine facies of the uppermost Messinian in the Balearic and Tyrrhenian Basins are probably time equivalent in part to the upper *Lago Mare* deposits of the Ionian and Levantine Basins. The latter were inundated after the seawater spilled over Sicilian Channel. The early Pliocene (Trubi) inundation probably did not reach the Red Sea which was separated from the Mediterranean by the Isthmus of Suez. The marine invasion there was dated as occurring some 3.5–4 Myr ago, when a connection to the Indian Ocean was established through the Strait of Bab el Mandeb³⁵.

Pliocene flooding and Plio-Quaternary subsidence

Leg 42A drilling confirmed the Leg 13 drilling results that the first Pliocene sediments above the Messinian are deep and open marine hemipelagic sediments. The Messinian salinity crisis was ended by the Pliocene flooding.

Palaeobathymetric analyses indicated that the Pliocene sediments immediately above the Mio-Pliocene contact are in excess of 1,000 m, or 1,500 m in depth. The drilling evidence proved that deep Mediterranean depressions which had existed before the Messinian salinity crisis were still there at the end of the crisis. Seismic evidence indicates considerable Plio-Quaternary subsidence³⁶. The subsidence is in part induced by the isostatic load of the water flooding the basins⁴. Additional subsidence could be related to cooling of the mantle under the basins, or to continued tectonic activities which may have deepened the Mediterranean back-arc basins³⁶. There have been considerable Plio-Quaternary tectonic activities in the circum-Mediterranean regions. The evaporite basins of Sicily, Calabria,

Apennines, Tellian Atlas, Ionian Islands, Crete, Cyprus, and so on, were uplifted and emerged. Meanwhile continental margins locally (Nile and Rhone deltas, Israeli shelf, and so on) underwent subsidence³⁷. Those activities have modified the post-Messinian palaeogeography. The fact remains, however, that the Mediterranean basins owed their genesis to earlier movements, even though their floor may have been uplifted or subsided somewhat during the last five million years.

Impact of the Mediterranean salinity crisis

The desiccation of the Mediterranean took place 5.5 Myr ago. Such a geologically recent catastrophic event left behind not only a giant evaporite deposit, it also had a great impact on the modern world, on the circum-Mediterranean landscape, on regional and global climates, and on the evolution and distribution of plants and animals.

The lowering of the base-level of erosion led to the deep incision of rivers which drained into the Mediterranean⁴; even the southern Alpine lakes may have been Messinian canyons modified by Pleistocene glacial erosion³⁸. The exposure of Mediterranean bottom promoted an unusually active Late Miocene groundwater movement, which was probably responsible for the initiation of karst topography in Yugoslavia and in other Mediterranean countries. The uneven topography of the Mediterranean Ridge may have been a modified karst-development⁴, anomalous drilling behaviour noted during Leg 42A drilling on the Ridge suggests presence of submarine caverns.

The salinity crisis induced a continued change towards a cooler and more arid climate in the circum-Mediterranean. The Antarctic ice sheet expanded greatly during the Messinian³⁹, and the Arctic ice sheets may have begun to form then⁴⁰.

Biologists are only beginning to explore the implications of the Miocene Mediterranean desiccation. The present Mediterranean fauna is similar to that of the Pliocene, but very different from that of the Miocene, a fact noted by Lyell more than 100 yr ago when he first established the Miocene and Pliocene Epochs. The periodic conversion of an inland sea into dry land also permitted a migration of land animals; numerous examples of exchanges between Africa and Europe during the Messinian have been found^{41,42}. With the sudden Pliocene flooding, animals stranded on the Mediterranean islands would become isolated and develop endemism^{41,42}. Other usual aspects of the present Mediterranean land faunas may also be related to the unusual Messinian event. We might ask, for example, if the unusual habit of the circum-Mediterranean eels to breed in the Mediterranean, rather than in the Sargasso Sea, might be traced back to the time when the gate to the Atlantic was closed. We might also invoke the Messinian *Lago Mare* expansion to explain the similarity of relic freshwater faunas in France, Spain, and North Africa to those of the present Caspian. The impact of the Messinian salinity crisis and the accompanying climatic changes must have greatly influenced the distribution of land plants. The aridity led to temporary or permanent expansion of savanna vegetations. The impact on plant-evolution is subtle, but intriguing. It was suggested that some of the Mediterranean floras (for example *Medicago*) evolved from perennial to annual forms so as to endure longer period of severe drought, and from cross- into self-pollinators because insects could not have followed the new hardy plants into the hot dry environment (K. Lesins, personal communication).

We close this article in a contemplative mood. Biochemical studies suggest that the African ape-human divergence took place 5 or 6 Myr ago during the Messinian^{43,44}, just about the time when what is known to be the earliest hominid lived in Kenya⁴⁵. Is the timing

purely coincidental, or did the great expansion of the East Africa savannas at the time of the Mediterranean salinity crisis promote hominid evolution? Is our very being today traced back to that unusual event?

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Catastrophic chemical events in the history of the ocean

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Catastrophic chemical events are characterised by sharp rises in $\delta^{34}\text{S}$ in the surface of the whole world ocean, and by greater overshoots locally. Three events are recognised and named for the formation in which they are most sharply displayed. The sharpness of the rise in $\delta^{34}\text{S}$ suggests that the sulphide deposition necessary to explain it must have been accumulating residual high- $\delta^{34}\text{S}$ seawater for some tens of millions of years out of contact with the surface ocean. A modified geological model is presented: brine generated by evaporite deposition is stored in deeps of a mediterranean basin; underneath the brine, pyrite precipitation builds a store of brine heavy in $\delta^{34}\text{S}_{\text{SO}_4}$, whose corresponding buildup of $\delta^{38}\text{O}_{\text{SO}_4}$ may balance the decrease of $\delta^{38}\text{O}_{\text{SO}_4}$ from evaporite deposition. Catastrophic mixing of the brine and the surface ocean, initiated by destruction of the storing basin, is the source of the sharp rise in the sulphur isotope age curve detected world-wide in evaporites. These events have important implications not only for modelling of the chemical history of the ocean, atmosphere, and sediments, but also for the explanation of faunal crises, and the many aspects of geology that depend on the composition and circulation of the oceans and their peripheral basins.

It has been known for some time that the sulphur isotope ratio in the world ocean surface has undergone major excursions during the period (Proterozoic-Phanerozoic) that could be documented from evaporite sulphate samples¹⁻³. The resulting sulphur isotope age curve has recently been updated with many new analyses of sulphur isotopes^{4,5}, supplemented by measurements of oxygen isotopes from some of the same sulphates^{5,6}.

The interpretations of these isotope age curves have focused mainly on explaining the trends of $\delta^{34}\text{S}$ as a result of widespread variations in biological reduction of sulphur into shales; on subsequent oxidative erosion into the sea; and with possible coupling of the carbon cycle to maintain atmospheric oxygen (refs 3, 5, 7-12). My purpose here is to discuss some more dramatic but less well-documented features of the isotope age curves, and to discuss whether they record catastrophic chemical events that are specific in time and place.

Upward jumps in $\delta^{34}\text{S}$

Fig. 1 shows as a solid line the mean best estimate for $\delta^{34}\text{S}$ in sulphate evaporite minerals in equilibrium with the surface of the world ocean^{4,5}. In constructing this curve greater weight was attributed to values whose world-wide distribution bore out their origin in the ocean surface as a whole, and less weight to results that were found in a narrow range of time and place. But it is worth noting that those of the latter deviations that are not so widespread may have considerable significance.

Marks on Fig. 1 highlight three rises in $\delta^{34}\text{S}$; and a solid line in Fig. 2 repeats these parts of the curve on an enlarged scale. These rises are so large and take place over such a short time, that I have labelled them chemical events by analogy with the nomenclature of magnetic reversals. Table 1 lists some characteristics of these three events. There are differences that may be significant, but some common features are: (1) The mean curve established by world-wide evaporite sampling rises sharply. (2) The rise is highly accentuated by a positive overshoot, in one formation in one basin, which gives its name to the event. The overshoot peak is a step function within the limits of stratigraphic control. (3) It is difficult to fix the absolute time interval within which the event is bracketed. Each seems to have taken place within one stratigraphic stage, probably much less than 5 Myr. (The times given in Table 1 are only scaled by inter-

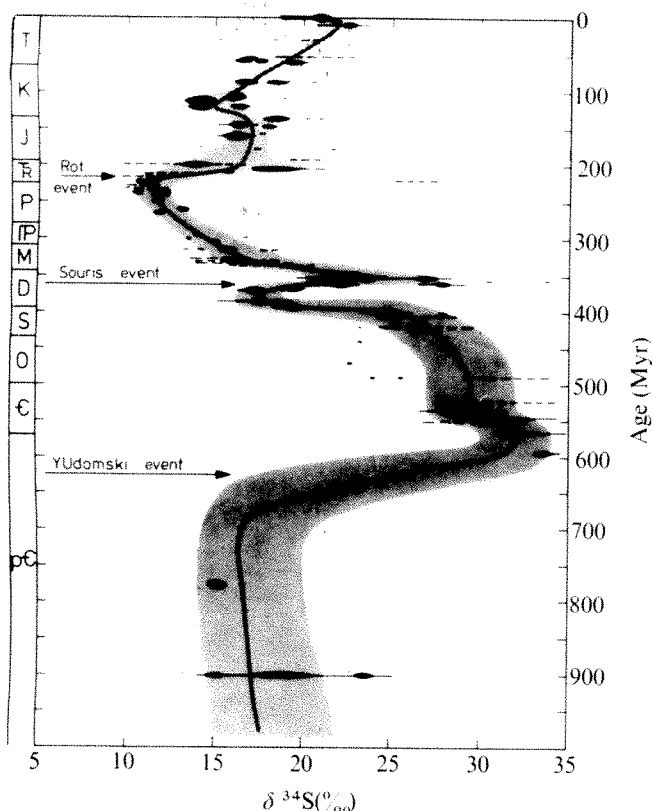


Fig. 1 Summary sulphur isotope age curve for marine sulphate, from Claypool, *et al.*^{4,5}, who give detailed plots and lists of analyses. Data are shown here by areas or lines that indicate qualitatively the number of analyses, plotted at their most probable age. The heavy line is the best estimate for $\delta^{34}\text{S}$ in equilibrium with the world surface ocean of that time; the shaded area is an estimate of the uncertainty of that determination. The named catastrophic chemical events are sharp rises in the best-estimate curve, accompanied by very high values ('overshoots') found only locally.

polation from radioactive dates that are for the most part separated by a whole geological period¹³.) (4) Each of the events is followed by a long period of mean oceanic $\delta^{34}\text{S}$ that is higher than the pre-event level, and of course lower than the overshoot peak. This post-event level is either constant (YUdomski and Röt Events) or relatively slowly decreasing (Souris Event) over a substantial period of time. (5) The mean curve for $\delta^{18}\text{O}_{\text{SO}_4}$ in

marine sulphates⁵ at equivalent times displays either no discernible rise (YUdomski and Souris Events) or a relatively small one (Röt Event).

Nature of the chemical event

Models for interpretation of the sulphur isotope age curve explain general rises as indicative of marine biological precipitation of sulphide predominant over weathering of sulphide, and falls as oxidative sulphide weathering (refs 4, 5, 8, 11, 12), although the models differ widely in assumed constants, residence times, and couplings. Processes of a similar nature must also explain the sharp events.

Detailed consideration of the isotope geochemistry of oxygen has led Claypool *et al.*^{4,5} to conclude that when $\delta^{34}\text{S}$ rises a corresponding rise in $\delta^{18}\text{O}_{\text{SO}_4}$ can only be prevented if the sulphide deposition is accompanied by extensive evaporite deposition. This is possible because with oxygen, much more than with sulphur, the isotope effect of evaporite deposition tends to offset the effect of sulphide deposition. Consequently, the chemical events seem to have involved major and contemporaneous deposition of sulphide into anaerobic muds and of sulphate into evaporites.

But these rises in the sulphur curve, and especially the local overshoots, are so precipitous that an extremely high rate of sulphide precipitation would be required to change the whole world ocean so much in so short a time. One can first of all see qualitatively that if the residence time of sulphur in the ocean (with cyclic sea salt subtracted) is about 20 Myr, as at the present time³, then it will be very difficult to generate changes of 5–15‰ in $\delta^{34}\text{S}$ within 5 Myr, and still more so in 1 Myr. Nielsen² calculated that the rate of change of $\delta^{34}\text{S}$ probably would not exceed 0.3‰ per Myr. In making this calculation, he estimated the present rate of sulphur erosion ($\approx$ deposition) of 15 Mt yr⁻¹, but the maximum rate would have meant the complete suspension of erosion to get the calculated rate of rise from sulphur deposition. This seems unlikely, especially as present rates of erosion may be higher than usual¹⁴. Nielsen had also to use the current value of the sulphur content of the ocean. It is true, however, that if the ocean were smaller, the same rate of sulphide deposition would raise $\delta^{34}\text{S}$ correspondingly faster. Holland¹¹ also assumed the present sulphur content of the ocean, as well as the present rate of sulphur input by rivers (uncorrected for cyclic sea salt and anthropogenic sulphur). His values of rises in available oxygen for the intervals that span my chemical events can be converted to amounts (of sulphur deposited) that are correspondingly large,

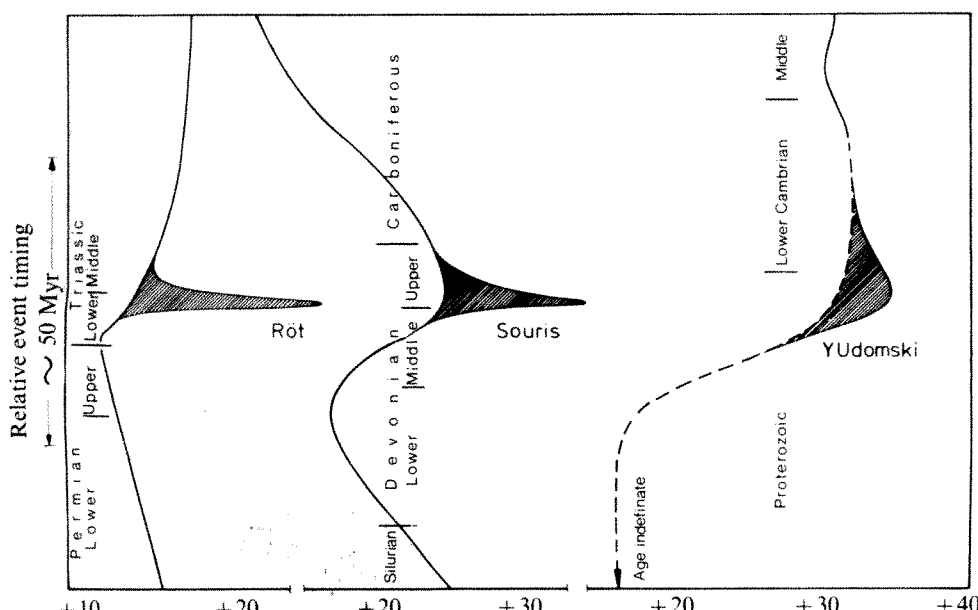


Fig. 2 The chemical events labelled in Fig. 1 are shown here schematically on greatly enlarged scale. Solid line: best estimate for $\delta^{34}\text{S}$ of sulphate mineral in equilibrium with world surface ocean; shaded area: local high overshoot. The timing is based approximately on stratigraphic stages, so that the absolute time scale is very approximate.

Table 1 Catastrophic chemical events

Suggested name Stratigraphic stage	YUdomski Event Pre-Aldan (Latest Proterozoic)	Souris Event Frasnian (Lower Upper Devonian)	Röt Event Scythian (Upper Lower Triassic)
Approximate age (Myr) Pre-event level $\delta^{34}\text{S}$	575	355	215
Stratigraphic stage	Late Proterozoic	Eifelian (Lower Middle Devonian)	Mittlerer Buntsandstein (M. L. Triassic)
Approximate age (Myr)	635–850	365	220
Mean $\delta^{34}\text{S}$, ‰	+15	+18	+13
Earliest peak in $\delta^{34}\text{S}$ Formation	YUdomski Horizon	Souris River and others	Röt
Location	Eastern Siberian Platform	Saskatchewan	Netherlands to DDR
Other localities	Saline Series, Pakistan	—	Moenkopi of Utah
Maximum $\delta^{34}\text{S}$	Hormuz Series, Iran		
Maximum excursion in $\delta^{34}\text{S}$	+35 +20	+34 +16	+27 +14
Post-event level $\delta^{34}\text{S}$ Stratigraphic stage	Lower to Middle Cambrian	Famennian (Upper Upper Devonian)	Muschelkalk (Middle Triassic)
Mean level	+30	+23	+19
Rise of mean $\delta^{34}\text{S}$	+15	+5	+6
Rise of mean $\delta^{18}\text{O}$	0?	+0.5	+2.5

and when compressed into an actual rise time even as long as 5 Myr, the rate of net sulphur deposition figures out to about 150 Mt yr⁻¹. Using the simultaneous solution of mass balances for both sulphur and oxygen isotopes I have also calculated similarly extreme rates of sulphide deposition. These calculated rates all seem unlikely, and suggest that the deposition of sulphide that was responsible for the rise in the age curve was accumulated over a long period of time, but was read into the evaporite record in a very short time.

The first evidence of the event is also the strongest peak, and in at least the YUdomski and Souris Events is found in only one basin. This suggests a chemical overshoot, possibly in the very area where the event was generated. The post-event level is seen on the whole ocean's surface, and the eventual decline corresponds to a more uniform decay through the weathering process.

A geological model

To explain the 'Permo-Triassic faunal crisis' (which is coincident with my Röt Event) Fischer¹⁵ suggested that brine was generated on shelves from evaporite or near-evaporite conditions, and then slid down as a density current to accumulate in the ocean deeps. This mechanism generates a stratified ocean that is at least temporarily stable, with brine below and a brackish surface layer. The characteristics of the isotopic events suggest the following modifications of Fischer's proposal.

Fischer required brine that was to some undefined degree dense enough to stably resist mixing; he allowed but did not require that evaporation proceed as far as evaporite precipitation. The oxygen isotope data indicate that evaporites of at least the calcium sulphate facies were generated in large amounts. The resulting brine, of density 1.10–1.20 × 10³ kg m⁻³ has therefore all the more likely to be hydraulically stable in the bottom of a basin. Other proposals^{16,17} have been based on evaporite deposition in the halite facies, and that is not precluded here.

Fischer suggests a deep-sea depression as the storage site for brine. That is not necessary, and it may not be correct. The bottom of any basin will do for brine storage, so long as it is large enough to hold the amount of brine that must be sequestered to account for the change of $\delta^{34}\text{S}$. A rift graben or other mediterranean has the advantage that it is more susceptible to the quick destruction suggested by the fast rise of the $\delta^{34}\text{S}$ curve, which represents chemical mixing of the deep brine and the surface layer.

The suggestion of a large basin in which evaporite deposition (as well as sulphide deposition) is important will probably remind the reader of the desiccating sub-sea-level basin^{18,19}, which

enjoys inflow from the ocean without reflux. One problem with that arrangement is that in conditions approaching desiccation, the required mixing of the brine is inhibited. Valiashko²⁰ has pointed out that even at the beginning of the potash evaporite facies, the volume of remaining brine may be accommodated in the porosity of the previously deposited salts. But the basin could be sub-sea-level, and non-refluxing, so long as it was not desiccating.

It therefore seems most likely that the site for brine accumulation is neither a deep-sea basin, nor a sub-sea-level desiccating basin, but rather a large mediterranean isolated from the ocean, especially with regard to its deeps.

A particular requirement for the chemical event is that evaporite deposition be accompanied by sulphide deposition, within the same basin complex if not actually the same basin. Although many evaporite sequences are grey and evidently associated with reducing conditions, the amount of sulphide is usually small. So it may be that the major part of the sulphide deposition occurs under brine accumulated nearby. Some present saline deeps support bacterial reduction of the large proportion of sulphate that they still retain, while others are sterile. The differences are not understood^{21–23}.

Sulphide deposition requires not only the generation of sulphide by sulphur-reducing bacteria, but also a sufficient supply of iron (ferrous or ferric) to fix the sulphide in the sediment. Not much sulphide can be retained in solution in the brine, and even this would be ineffective for the chemical event if it were simply remixed with the ocean. Although sulphide deposition in ordinary bottom muds is usually supposed to get its iron from the sediments themselves (probably from hematite or goethite coating sand grains)²⁴, the amount required for building the large reservoir of fixed sulphide suggests that supplies could well be augmented from a more plentiful source such as submarine volcanism^{25,26}.

According to Fischer¹⁵ the fossil record suggests that during the Triassic the surface ocean returned to normal salinities. The sulphur isotope record also suggests this—but the sharp onset indicates a more catastrophic mixing of brine and surface ocean. At that time, in addition to the restoration of normal salinity described by Fischer, we may have a catastrophic inflow in various places of waters rich in salts, organic matter, sulphide ions, nutrients, oxygen demand, and $\delta^{34}\text{S}$. The local nature of the overshoot in $\delta^{34}\text{S}$ is further evidence for the catastrophic nature of the mixing. The location of such an overshoot suggests but does not necessarily prove that the brine was stored in a basin nearby.

In accordance with current custom much of the above can be stated in terms of the theory of plate tectonics. The rifting Atlantic is prominently associated with evaporites (refs 19, 27

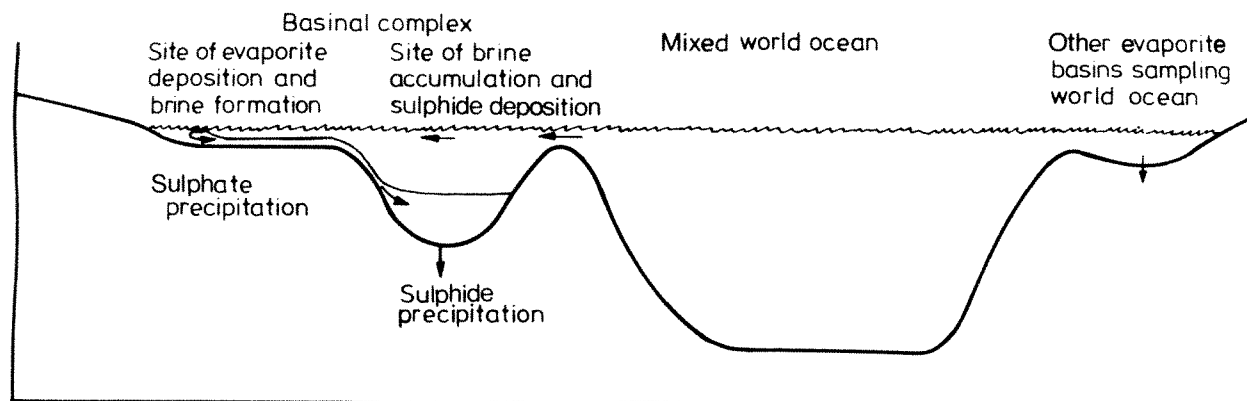


Fig. 3 Geological model to explain the chemical events, somewhat modified from Fischer¹⁵. A basin or basin complex precipitates sulphate, and generates and stores brine, under which reduced sulphide is precipitated. The evaporites of the world (both left and right) sample only the surface ocean, out of contact with sulphate residues of the sulphide reduction, until its accumulated effect is catastrophically mixed with the surface waters at the time the basin complex is destroyed.

and 28); and Kinsman (refs 17, 29 and 30) has generalised and explained this association in the following way: rifting leads not only to initial rift valleys like the Red Sea, but also continuously generates subsiding continental margins, as well as transverse tensional grabens. These are all potential sites of restricted circulation and evaporite deposition along the trailing continental edge. Isolation may be aided by island-forming volcanism in the rift. Kinsman¹⁷ also pointed out the fast timescale of evaporite generation on specific sections of a continental margin—his time limit of 10 Myr is compatible with the chemical event reported here. I need only add to this description that brine sliding down and accumulating in the rift basin will help reduction of sulphide, and volcanic activity or associated thermal waters may supply iron and other heavy metals to fix the sulphide in giant deposits^{25, 26}. Eventual full separation of the continents mixes brine and surface ocean. This scenario is a possible one, but other tectonic situations may also generate the required structures and geography.

History of a chemical catastrophe

The story line is indicated in Fig. 4. At time *A* we find the sulphur isotope age curve slowly declining as a result of erosion of predominantly sulphide material into a mixed ocean. This erosion exceeds the sulphide deposition. At time *B*, dense residual brines from evaporite deposition begin to accumulate in the deep of a large nearby basin. Reduction to sulphide continues and $\delta^{34}\text{S}$ in the brine gradually begins to reverse and then increase, while at the same time its mass continues to grow as a larger and larger proportion of the ocean's sulphate is processed through the sulphate-sulphide production system. Meanwhile, the surface layer becomes more brackish, and its $\delta^{34}\text{S}$ continues to fall under the influence of the continuing erosion of older sedimentary sulphide. At point *C* the accumulated brine with high $\delta^{34}\text{S}$ begins to leak or mix slightly with the surface body of the ocean, slowing and even reversing the downward trend of $\delta^{34}\text{S}$ recorded in evaporites everywhere. At point *D* the climax of the event is reached as the brine is released, perhaps through tectonic destruction of the basin. Local appearance of the high $\delta^{34}\text{S}$ may be sampled by evaporites as an isolated record of $\delta^{34}\text{S}$ overshoot. Mixing of brine and surface water leads to an intermediate level of $\delta^{34}\text{S}$ in the subsequent evaporite sampling of the now mixed ocean. As erosion assumes greater importance again, at point *E*, $\delta^{34}\text{S}$ begins to decrease. This may continue until the required combination of evaporite production and basin geometry again induces brine accumulation.

Point *A* does not leave a record that I know how to recognise, so I don't know how long before the event brine segregation and accumulation began. It is conceivable that all the depressed sections of the $\delta^{34}\text{S}$ curve represent segregation and that therefore erosion never really takes over, but only becomes more evident when its depositional counterpart is hidden in a deep.

I realise that the explanation proposed for the chemical events is only a skeleton, and I exhibit it here so that others may join me in either giving it flesh or burying it. Whether it is viable or not the events are real and demand another explanation if not this one.

Geological model

Each of the events displays some individual character in the isotope age curves—these will now be described. And to follow up on the conjecture that the location of brine storage is flagged by the overshoot, the palaeogeography will be examined for conditions favourable to brine accumulation and sulphide reduction.

Yudomski Event. This event occurs just below the boundary of the Proterozoic and the Cambrian, between the Shaler Group of the Arctic Islands that can only be bracketed at 630–850 Myr (ref. 31), and the Yudomski Horizon of the Siberian Platform that is said to be dated at 600 ± 10 Myr (ref. 32). So the abruptness of the rise shown in Figs 1 and 2 is drawn by analogy to the later two events. Within the vague limits of the available data, $\delta^{18}\text{O}$ is unchanged throughout the whole event⁵. The level $\delta^{34}\text{S} \approx 17\text{‰}$ in the Proterozoic pre-event stage is puzzling. It could be an artefact of an imperfect record. Perhaps brine was accumulating all that time, leading to the climax of the chemical event at the Cambrian boundary. But in that case one would have expected the surface ocean as sampled by the evaporites to have shown a declining curve due to erosional input.

The earliest record of this event is an overshoot to 33‰ in our new analyses of the Yudomski or Motskaya Suite, which crops out around the southern edge of the Irkutsk Amphitheatre in Siberia. This horizon lies comfortably below the Cambrian³³, which especially in its middle stage developed into one of the world's largest evaporite basins²⁴. There are earlier evaporites, as yet unanalysed for isotopes, in Siberia that precede the Yudomski³². All of these Siberian basins opened in the direction of the present Arctic, and that could have been the locale for brine accumulation, but its palaeogeography and tectonics are unknown to me.

A possible alternative site for the main event is suggested by one analysis of high $\delta^{34}\text{S}$ in the Cambrian of the Salt Range of Pakistan⁶; this horizon evidently overlies the extensive Saline Series of possible Late Precambrian age³⁵, which may also be correlative with the very extensive Hormuz Series evaporites that extend across southern Iran^{36, 37}. This has been confirmed by unpublished high values of $\delta^{34}\text{S}$ obtained by Heimo Nielson (personal communication) from several samples of the Hormuz Series.

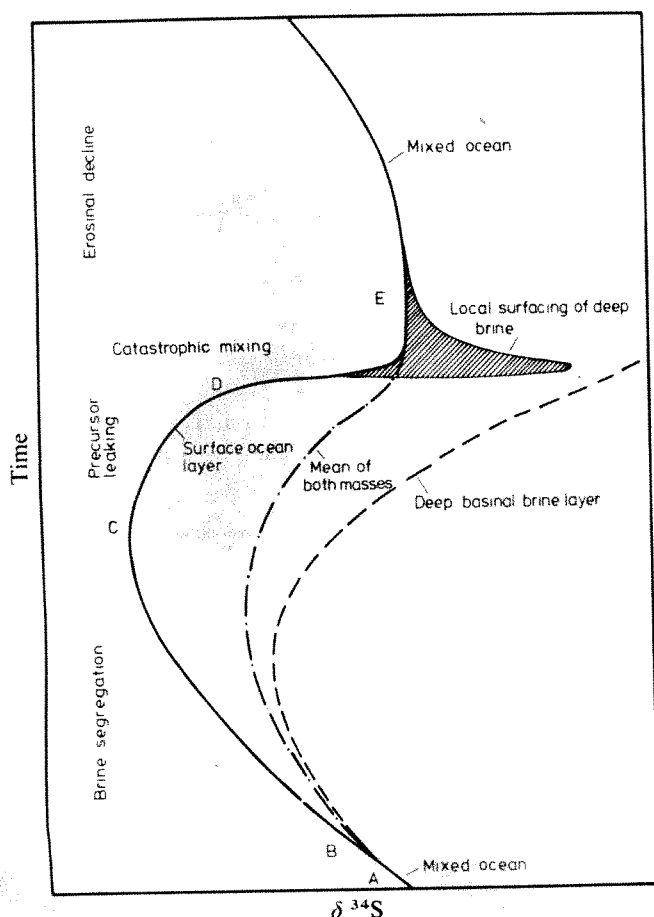
Souris Event. A special character of this event is a well-documented gradual rise in worldwide $\delta^{34}\text{S}$ during the Middle Devonian, that precedes the sharper rise to the peak in the Famennian Stage^{5, 7}. In terms of the model, this represents a more gradual leak or mixing from the stored brine into the ocean.

A strong overshoot is displayed in the Souris River Formation and related beds near the top of a giant sequence of evaporites that accumulated in the Elk Point Basin of Western Canada, beginning in the Middle Devonian. The basin was recharged from the north, across the strongly developed Presquile Reef in the North-west Territories. Beyond the reef black marine shales were extensively deposited³⁹, and this area may have been the place of brine accumulation and sulphide reduction. It lies at the edge of the Eurasian basin, whose palaeogeography and tectonics are still controversial^{39,40}.

Röt Event. This event occurs sometime between well-established low levels of $\delta^{34}\text{S} = 11\text{‰}$ in the latest Permian, and the moderate levels of the Middle Triassic and later Mesozoic stages. Although in many areas stratigraphic subdivision of the Lower Triassic has not been successful, analyses from the well divided Buntsandstein of East Germany⁴¹ and West Germany (unpublished data by Dietrich Rambow and Heimo Nielsen) show that low values of the Permian continue through the Lower and Middle part of the Lower Triassic.

The Buntsandstein exhibits a most dramatic overshoot, up to $\delta^{34}\text{S} = +27\text{‰}$, in the Röt (Upper Lower Triassic), whose evaporite facies extend from Poland across Germany (East and West) into England^{42,43} and touch Greenland⁴⁴. The main

Fig. 4 Schematic representation of sulphur isotope effects during the course of a chemical event. At the earliest time, *A*, $\delta^{34}\text{S}$ is slowly decreasing as erosion of sulphide exceeds deposition of sulphide. At *B* brine starts to accumulate in a basin complex as a result of evaporite deposition (Fig. 3), and biological reduction under the brine begins to slow and then reverses the decline of $\delta^{34}\text{S}$ in the residual sulphate in the brine, but out of contact with the surface ocean which continues to decline in $\delta^{34}\text{S}$ (as sampled by worldwide evaporites). At *C* the brine begins to mix somewhat with the surface ocean, and at *D* mixing becomes catastrophic, with local overshoot due to surfacing of deep brine. At *E* a level for the now mixed ocean is a weighted mean of the previous two reservoirs of sulphate; the course of this fictive mean isotope composition during the event is shown by the middle line.



development of Röt evaporites coincides with much of the thickest Zechstein evaporites. If, as seems plausible^{42,43}, the Röt and Zechstein had a common entrance to the north ('Skandik Sea'), this might furnish a suitable site for brine accumulation; although an alternative opinion is that the Röt was open to the Tethys Sea to the south-east⁴⁵.

A few analyses⁵ suggest a possible alternative site for this event, in the Lower Triassic areas of the Western United States.

Note that all three events have possible connections with brine accumulations in the region of the Palaeozoic geography that lies in the present Arctic.

Comparison with other theories

Thode and Monster¹ proposed that certain basins would have high values of $\delta^{34}\text{S}$, due to greater amounts of sulphur reduction, and they therefore assumed as a value for the world ocean the lowest $\delta^{34}\text{S}$ in any basin at a given time. In the present instance reduction in a basin is also proposed, on an even larger scale, but its residues remain out of contact with the world ocean surface until the catastrophic event of mixing.

Pilot *et al.*⁴¹ dealt specifically with the Röt event: They proposed that an accumulation of sulphide reduction in the Tethys Sea had been going on since the Carboniferous, and that the Röt evaporites were drawn from that sea, in contrast with the Zechstein which was drawn from the northern sea. For my model it is not sufficient to isolate Tethys and deposit sulphide there so that the Röt can tap it with a high $\delta^{34}\text{S}$ evaporite. The sulphide-depositing basin also has to derive a large amount of sulphate from the world ocean, while at the same time holding back its high $\delta^{34}\text{S}$ until a critical moment. I accomplish this with the evaporite-generated brine layer. This could have happened in Tethys, but for the reasons described above I think the northern sea is more likely.

Other speculations concerning the history of the ocean, while not specifically directed towards the interpretation of isotope age curves, are related to major chemical events. Buerlen¹⁶ proposed that the faunal crisis at the Permian-Triassic boundary might be connected with a decreasing salinity of seawater due to the extensive precipitation of evaporites during the Permian. As indicated above, Fischer¹⁵ had a similar explanation for this palaeontological problem, but made the surface ocean brackish by the accumulation of deep brines rather than necessarily by deposition of salt. His paper was the real ancestor of this one, but its significance was not appreciated until the isotope data had been accumulated in sufficient detail. Kinsman¹⁷ showed how the kind of events proposed by Buerlen (although he did not refer to either Fischer or Buerlen) may have been very strong during the early phases of a continental rifting. None of these proposals connected the generation of brackish surface waters with sulphide reduction, which is the main point of the new proposal. More recently, Degens and Stoffers⁴⁶ proposed that density stratification, of unspecified origin, may have been a common phenomenon in earlier seas, on the model of the present Black Sea. Reducing conditions in the brine and the sediment, and accumulation of organic material, are important aspects of the model of Degens and Stoffers. What I am adding to their model is an emphasis on sulphide deposition in the sub-brine sediment, a suddenness of re-mixing with the surface ocean, and a method of specifically detecting such situations in the geological record by the interpretation of the isotope age curves.

Catastrophic chemical events—implications for future work

(1) Modelling of ocean history and the calculations of oxygen demand (positive or negative) associated with sulphide-sulphate transfers will be more complicated than it has been^{4,11,12} under the assumption of a mixed ocean. In principle it will be possible to make new calculations with the sea sulphate divided between two reservoirs, surface and brine. Even without such calculations, one can say qualitatively that the overall decrease of $\delta^{34}\text{S}$ in the pre-event stage will be less

than that for the surface ocean alone, so that the maximum oxygen demand associated with a $\delta^{34}\text{S}$ minimum will be less than would have been calculated assuming a mixed ocean.

(2) The dominant sulphide reduction during the pre-event stage will be under the brine reservoir. Therefore during the deep minimum in the $\delta^{34}\text{S}$ evaporite curve, a corresponding $\delta^{34}\text{S}$ curve for these particular sulphides need not show such a deep minimum or, indeed, any minimum at all. Whether sulphides of the briny deep are mainly in black shales, as seems most likely, or also in large stratiform sulphide deposits, remains to be determined. The limited sulphur isotope data presently available for both styles of deposition have been reviewed by Schwarcz and Burnie⁴⁸, but these represent only a handful of occurrences.

(3) The combination of evaporite deposition and brine formation decreases surface salinity (in all its components) of the ocean during the pre-event phase. The surface ocean should also show a corresponding increase of δD and $\delta^{18}\text{O}_{\text{H}_2\text{O}}$, which should be looked for in fossils, limestones, and cherts, but on a finer time scale than previously tried^{15,48}.

(4) The decrease of sulphate in the surface ocean may allow the buildup, from erosional input, of elements such as barium and strontium; they may be precipitated during the subsequent mixing. Some sedimentary barite deposits^{49,50} coincide exactly with the Souris event; and peaks of strontium in shales are found near the YUdomski and Röt Events⁵¹. The age curve for $^{87}\text{Sr}/^{86}\text{Sr}$ shows a number of sharp rises⁵²⁻⁵⁴, and each of the chemical events postulated here has one of these jumps in $^{87}\text{Sr}/^{86}\text{Sr}$ associated with it: YUdomski Event—from 0.7075 in the Late Proterozoic (570 to 850 Myr?) to 0.7095 in the Lower Cambrian; Souris Event—from 0.7080 in the Givetian and Frasnian to 0.7093 in the Famennian; and the Röt Event—0.7073 in the Kazanian and Tartarian to 0.7083 in the Early Triassic. Veizer and Compston⁵³ noted that the fine structure of the strontium isotope curve might "... indicate that the fluctuations in Sr isotopic composition of seawater could develop within several million years." But the difficulty here is similar to sulphur—a residence time in a mixed ocean that is much longer than that.

(5) The possible effects of these various chemical changes on biological processes, ecology, and evolution may be far-reaching. Some of these have already been discussed in earlier models: pre-event increase in oxygen demand¹¹ (but less than previously assumed¹²), and decrease of surface salinity¹⁵⁻¹⁷. The new model would generate the added stress of a quick return to more or less normal salinity. The remixing of anoxic brines into the ocean may have profound effects, particularly in local regions of overshoot. Nutrients may be removed from effective participation in the biological cycle—temporarily with all seawater constituents, or by sedimentation under the brine. In the post-event stage re-mixing restores to the productive surface waters those nutrients still in solution, with consequent effects on organic productivity and possibly increased deposition of organic carbon and decrease of atmospheric $p\text{CO}_2$. A post-event increase in phosphate in surface waters may account for the proliferation of apatite-secreting metazoa in the Cambrian⁵⁵.

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letters to nature

Future variation of Planck's constant

RECENT interest in cosmological models involving time-varying fundamental constants has stimulated experiments to measure such variations which may have occurred in the past. For example, the upper limit to past variations of h relative to other

fundamental constants has been measured as one part in 10^{12}yr^{-1} (refs 1 and 2). The purpose of this letter is to point out that the results of an experiment already performed by Partridge³ for a different reason, can be interpreted as setting a limit to the future variation of h over cosmological times.

In the quantum absorber theory of Hoyle and Narlikar^{4,5},

based on Feynman's path integral approach with a constant $\hbar$, it is the quantum mechanical response of the future part of the universe, rather than local field quantisation, which gives rise to spontaneous emission. This indicates the possibility of measuring future universal behaviour by experiments conducted in the present.

The method of modifying any form of quantum mechanics to allow for a hypothetical variation of $\hbar$ is of course not established experimentally. We can, nevertheless, proceed with reasonable postulates to generalise quantum absorber theory as follows. Consider a charged particle a at time t_0 , which is part of a quantum system such as an atom, in interaction with a similar particle b at some large distance away at a different time t_1 . If the interaction is negligible the quantum behaviour of a is determined by the dimensionless quantity $S_a/\hbar(t_0)$, where S_a is the free action for a and $\hbar(t_0)$ is the value of $\hbar$ at times near t_0 . (We assume the variation of $\hbar$ is small enough for $\hbar$ to be regarded as constant over short laboratory time scales.) A similar quantity $S_b/\hbar(t_1)$ determines the free behaviour of b . When the interaction is not negligible, an interaction term S_1 must be added to the action, and the question arises as to what value of $\hbar$ should be used with this term. In a completely time-symmetric universe some symmetric quantity such as $\frac{1}{2}(\hbar^{-1}(t_0) + \hbar^{-1}(t_1))S_1$ would seem appropriate, but in an asymmetric universe, in which only the future part provides a perfect absorber, it seems preferable to be guided by the self-consistent cycle of argument of Hoyle and Narlikar⁵ in which only retarded fields are used to calculate interactions. Thus if $t_1 < t_0$ the interaction is calculated from $-e\int_{t_0}^{\infty} \hbar^{-1} \mathbf{A}_b^{\text{ret}}(\mathbf{a}) \cdot \dot{\mathbf{a}} dt$ where $\mathbf{A}_b^{\text{ret}}(\mathbf{a})$ is the retarded field of b acting on a at position $\mathbf{a}$, and, since the integral is taken over an interval near t_0 , it is reasonable to take $\hbar = \hbar(t_0)$. On the other hand when $t_1 > t_0$, as it is when b is one of the particles of the absorber, the appropriate expression is $-e\int_{t_1}^{\infty} \hbar^{-1} \mathbf{A}_a^{\text{ret}}(\mathbf{b}) \cdot \dot{\mathbf{b}} dt$ with $\hbar = \hbar(t_1)$.

The values of $\hbar$ can be used to find the influence functional containing the quantum mechanical response of the Universe. The dependence of this functional on $\hbar(t_1)$, the value of $\hbar$ at the time of absorption, arises from the free action terms of the absorber and the interaction terms, and gives the way in which the lifetime of atom a in this modified theory depends upon the epoch at which absorption takes place. An experiment which can compare the transition rate in a source when the source is emitting into a local absorber, for which $\hbar$ takes its present value $\hbar(t_0)$, with the rate when the source is emitting into an absorber at a much greater distance where absorption takes place at time t_1 in the future, would thus give a measure of the future variation of $\hbar$ over the time scale $t_1 - t_0$. Just such an experiment has been done by Partridge³. In this experiment microwaves from a source were alternately directed into a local absorber and into the Universe. For the latter case Partridge estimated that at least 95% of the radiation would reach cosmological distances before absorption. The transition rate from excited states in the source was monitored by measuring the power needed to re-excite the source. Assuming a relation $P_1 = (1 - \delta)P_0$, where P_0 and P_1 represent the power used by the source in the presence and absence of the local absorber respectively, Partridge obtained a value $\delta = (1.1 \pm 1.6) \times 10^{-9}$. Although this experiment was performed for the different purpose of testing the completeness of the universal absorber, and the success in achieving this purpose has been questioned⁶, we can nevertheless use Partridge's data here. Writing $\hbar(t_1) = (1 - \Delta)\hbar(t_0)$ with Δ small, and using $\hbar = \hbar(t_1)$ in the influence functional determining the transition rate, we find that $0.95|\Delta| \approx |\delta|$. Taking the cosmological distances involved to be between 10^9 and 10^{10} light yr at least, gives an upper limit to the variation of $\hbar$ of about one part in 10^{18} yr⁻¹.

This result, which must be one of the longer time constants recorded, would be modified if, for example, the symmetric form of $\hbar$ mentioned earlier were used in the interaction term. The importance of the measurement however, lies not so much in the actual numerical result but in the fact that it is a measurement of the future variation of a physical quantity. It is important to

emphasize that this is not a prediction of future behaviour but, assuming the validity of the theory, is an actual measurement of behaviour which will occur. The apparatus 'sees' into the future.

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Astrophysical X-ray spectra: alive and well

CRAIG and Brown¹ have repeated their doubts about the usefulness of high resolution X-ray spectra² and reiterated a series of inaccurate and misleading statements. For example, as early as their second sentence they incorrectly claim that "a high resolution spectrometer is almost invariably included in solar X-ray satellites". High spectral resolution can only be achieved with a collimated spectrometer, the total useful observing time of all such rocket borne devices being currently of the order of an hour. But no solar satellite has ever carried such a spectrometer and only one such device is planned to be in orbit before 1980.

Contrary to the impression given by Craig and Brown, high resolution solar X-ray spectra have found a wide application in the fields of solar physics, atomic physics and astrophysics. High resolution spectrometers are clearly required to resolve line blends, with spectral resolutions of $\lambda/\Delta\lambda \sim 10^3$ being required in some instances (ref. 3 contains several examples). When reliable line intensities have been obtained the atomic physicist has an abundant source of material. In the well known cases concerning Fe XVII (refs 4, 5) and the Helike ion satellite lines⁶⁻⁸, high resolution observations have stimulated formulation of more accurate theories, and progress on many other poorly understood ions is held up by lack of sufficient spectral resolution.

Abundances are fundamental astrophysical parameters that have also been derived from X-ray spectra, not by model-dependent methods as Craig and Brown mistakenly state, but by using line ratios which are insensitive to temperature⁹⁻¹¹. For future experiments further improvements in spectral resolution are planned so that true line profiles can be measured and line widths and shifts investigated.

The main problem worrying Craig and Brown concerns the expression for the intensity of a single spectral line in terms of a Fredholm integral of the type

$$p(a) = \int q(b) r(a,b) db$$

Such integrals occur frequently in astrophysics and the problems associated with solving such equations have been extensively discussed. Probably the most commonly encountered form of the above equation occurs in radiative transfer theory when expressing the emergent intensity of a stratified atmosphere in terms of the source function. The elementary approximation of a linear dependence of the source function on the optical depth has little physical significance. Neither does the Eddington-Barbier relationship which is subsequently derived, but the usefulness and accuracy of this end result cannot be disputed.

Given the inherent mathematical restrictions relating to the form of the above equation, progress on the astrophysical problem can only be made by adopting one of the following

procedures: (1) The use of a sophisticated matrix inversion technique, which helps to overcome the problems of 'bad' data and a limited range of observations, for example, Phillips-Twomey, Backus-Gilbert. (2) The derivation of a 'best fit' model with no *a priori* assumptions about its structure^{3,12,13}. (3) The derivation of a 'best fit' model with a predetermined analytical structure¹⁴⁻¹⁶.

At present solar X-ray observations are not of sufficient quality to justify the use of procedure (1). There is nothing inherently wrong with a 'best fit' model provided its limitations are recognised and it is used sensibly in its relevant domain. In fact most astrophysical models have been constructed using either (2) or (3) above: only the simplified situations seem to have exact solutions. Thus 'best fit' active region models are presently no less accurate than many other astrophysical models and they do have every prospect of improving when observations of more lines covering a wider temperature range become available.

Craig and Brown have used an elementary simulation to predict the intensities of several lines for an assumed structure. These line intensities are given a random perturbation and a model is computed. However, they overlook the fact that emission measures are everywhere defined as positive quantities. The problem of negative emission measures arises from using pairs of lines with broadly similar temperature responses such that small perturbations in the data lead to inverted flux ratios. Whilst it is true that none of the computed models seem to resemble the input structure, a simple averaging of these models, that is, a 'best fit' (or at least a 'better fit') gives a model closely resembling the input structure, indicating that a convergent (self-consistent) solution to the problem is possible.

In conclusion, it is safe to say that the interpretation of X-ray spectra is alive and well, and will continue to thrive with improved instrumentation.

'Best fit' models for solar active regions are just as accurate as any other astrophysical models and far from being 'spurious' are found to be in agreement with others derived from ultraviolet and optical data. X-ray astronomy in general, after several initial breakthroughs with 'quite simple detectors' will continue to make progress with telescopes having improved spatial resolution and spectrometers with improved spectral resolution.

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those involved in space experiments, but their statements represent only one extreme viewpoint on this complex problem.

The main thrust of their argument is to point out the ill-conditioning of the equations that require to be inverted in order to carry out an analysis based on emission measures, and to illustrate this by reference to a 1970 publication by Batstone *et al.*³, an early attempt to do this in the X-ray region. It should be pointed out that emission measure analysis is only a small (though important) part of the full capability of spectroscopic diagnostics; that nowadays it is possible to combine it with spatial and time resolution of the source, and that the 1970 work is in any case not as unsound as they suggest.

To start with the last point, it is basic to all interpretational physics that the aim is to find the simplest model consistent with the observations. To insist, as do Craig and Brown, that all possible models, however complex, are equally valid and worthy of consideration would have seriously impeded progress in any branch of physics to which it had been applied. If we consider the histogram of emission measure X against temperature T from their example in terms of its Fourier components in harmonics of the temperature parameter, then it is clear that the simplest model is one with only low frequency components. The solution derived by Batstone *et al.*³ is a monotonic decrease from $T = 1$ to $T = 10$. Craig and Brown's alternative valid solutions all have for their largest amplitude Fourier component the highest harmonic permitted by the temperature interval— X oscillates violently up and down between each temperature point. Most physicists will recognise the unique nature of the simplest solution proposed by Batstone *et al.* in contrast to the contrived complexity of the alternatives. Furthermore, if we apply a simple smoothing (averaging pairs of adjacent points) all four models become very similar. Thus if we remove the highest Fourier component (normally spurious when fitting data based on discrete intervals) the physical reality of what is left is manifest by the fact that Craig and Brown's models, derived specifically to demonstrate the variability, tend to approach the same solution. The exception is the lowest temperature point, which remains variable to some extent due to the simple physical fact that the experiment is not very sensitive at these low temperatures. Craig and Brown, although right in demanding some form of error estimates in published data, are wrong in assuming that the full curve with all its temperature points is required in order to derive any significant conclusions from the data, and are also wrong in claiming that a single source temperature and variance could equally well describe the model. The significant data from this particular spectrum is probably describable by at most three points on an X against T curve, with the assumption of monotonic variation between them, and an asymptote at high T of zero. This is perhaps the limit of the information content, but such information can be very valuable. With modern space instrumentation techniques, data of this type can be obtained for a solar structure as a function both of position in two coordinates, and of time. Such a matrix of observations would provide a formidable challenge in the fitting together of theoretical and observational models. In practice, such a measurement nowadays would be likely to cover a much wider range of temperatures, and thereby further enhance its usefulness.

Emission measure analysis has long been used in the ultraviolet region for solar (and stellar) chromosphere structure determinations³. I have drawn attention⁴ to the limitations of this technique. In the ultraviolet case, however, the technique is much more precise than for X rays from active regions, and the limitations referred to above relate only to fine detail in the X against T curve. Craig and Brown claim that the method is always ill-conditioned due to the broad form of the kernel function F ; or to use the more usual Pottasch⁵ notation, the temperature dependent line factor $g(T)$. They claim that this is because F is always dominated by the Maxwellian electron distribution factor, $\exp(-E/kT)$. In this they are wrong. F contains the ion stage population N_i/N_e which is built up from

Why measure astrophysical X-ray spectra?

CRAIG and BROWN¹ advise caution against some of the limitations of X-ray astronomy, and emphasise the need to take theoretical considerations into account before planning expensive experiments. Their remarks must be taken seriously by

the product of many functions of the type $\exp(-E_i/kT)$ and consequently falls off much faster than a single function of this type, and in a way which varies with the wavelength region and type of ion chosen.

The above situation can be recognised more physically if we make an approximation to equation (1) of Craig and Brown

$$I(\epsilon) = \int_T \xi(T) F(\epsilon, T) dT \quad (1)$$

If the functions are transformed to a logarithmic temperature scale, so that $\theta = \log T$, then the kernel $F(\epsilon, \theta)$ can usually be approximated by a constant-shaped function, centred on a temperature θ_ϵ which is a function of the particular line ϵ . This is particularly true for the case dealt with by Batstone *et al.*², in which the lines are mostly isoelectronic, that is, they have similar atomic parameters, excitation energies and so on in relation to the charge on the ion. The equation can then be rewritten

$$I(\epsilon) = \int_\theta \xi(\theta) F(\theta - \theta_\epsilon) d\theta \quad (2)$$

Equation (2) can be recognised as a simple convolution integral in which the observable $I(\epsilon)$ is the result of folding the unknown $\xi(\theta)$ with a constant 'instrumental' profile $F(\theta)$. Its inversion properties are well-known; $\xi(\theta)$ can be derived from $I(\epsilon)$ reliably in regard to structure which is on a θ scale large compared with the width of $F(\theta)$, but becomes very ill-conditioned when trying to extract detail on a smaller scale than the width of $F(\theta)$. This simple approximation is useful to the physicist in understanding when the inversion of equation (1) is valid, and the inherent limitations in the solution obtained.

I now add a few remarks in defence of high wavelength resolution spectroscopy. The separation with confidence of spectral line blends into discrete lines opens up wide possibilities for the use of line ratios which, through the physics of the excitation mechanism, can be interpreted in terms of local electron temperature and density. This method can be much more precise than emission measure analysis, but is at present limited in its scope by limitations in the available atomic collision rates. The excitation process has a shorter relaxation time-constant in the plasma than emission measures which depend on ionisation balance. Consequently, the combined use of line ratios and emission measures can give vital information on the transient state of the plasma, that is the rate at which it is ionising or recombining. For solar X-ray measurements nowadays, lines and continuum can usually be separated. But, because of the lower fluxes, cosmic X-ray measurements so far have generally been broad-band, using the proportional counter as a spectrometer. Consequently, the expected strong line groups appear only as a bump in the spectrum, and it may be suspected that many of the 'continuum' measurements are contaminated by un-resolved lines. To suggest as do Craig and Brown that such crude detectors are more than adequate is therefore incorrect. When a future generation of cosmic X-ray spectrometers is able to resolve and separate lines, a whole new dimension of diagnostics will become possible and we can look forward to dramatic advances in our understanding of the recently discovered objects.

In conclusion, the field of spectroscopic diagnostics of space plasmas has come a very long way since 1970, when the emission measure analysis of a source, smeared over space and time, was a new development. We can now make detailed and precise measurements of a range of physical properties. Unfortunately, this calls for complex and sophisticated observational techniques, involving high spectral, space, and time resolutions and increased sensitivities. The criticisms levelled at emission measure analysis by Craig and Brown are not all sound, and ignore the proper role of such techniques within a broader

base which includes a range of more sophisticated techniques. Space experiments are not nowadays designed and built without input from theory. Most of the consortia currently in this field include members well versed in the present status of theoretical models, and with a broad understanding of the range of sophisticated diagnostic techniques currently available.

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BROWN AND CRAIG REPLY—We do not refute the value of very high resolution spectrometry to atomic physics, nor in astrophysical abundance and line profile studies. However, grant proposals for satellite spectrometers of $\lambda/\Delta\lambda \approx 10^3$ usually identify $\xi(T)$ measurement as a primary aim (recognising that atomic line identification is harder to justify in an astronomy budget). Concerning the inference of $\xi(T)$ we reply:

Simplicity of models?—Gabriel's point of smoothness in $\xi(T)$ is well taken but a half sine wave in $(1-10) \times 10^6$ K fits the data and is simpler (one Fourier term less) than his "unique simplest solution" without remotely resembling it. It is also positive everywhere, which may please our critics, but so are about 500 other diverse meaningless solutions we generated¹ which fit the data. Strangely both authors claim convergence of our $\xi(T)$ from three random terms² when we proved¹ non-convergence after 10^4 terms! We agree with Parkinson that AR analyses may be "no less accurate than many other astrophysical models" and indeed recommend close scrutiny of the non-uniqueness of all such. Astrophysical plasmas are regrettably unaware that they should be smooth and simple^{4,5}.

Validity of analysis?—Gabriel's reference to convolutions and Parkinson's to radiative transfer integrals in fact amply vindicate our analysis. The extreme width of $F(\theta)$ in the former proves⁶ that $\xi(T)$ can only ever be crudely resolved so that high spectral resolution is futile for this purpose. Would Gabriel kindly reference one line whose temperature response width $\Delta T \ll \epsilon_{line}/k$? Parkinson correctly states that radiative transfer theorists regularly use linear source function approximations but they always emphasise that this crude result cannot be much improved by increasing data resolution (ref. 7 and J. T. Jefferies and Skumamich, personal communication) (for example more limb darkening coefficients)—precisely our point. We are well aware⁶ of the 'more refined' data reduction techniques possible but our critics seem unaware that these all heavily use *a priori* assumptions about the solution to augment the low information content of the experiment^{6,8}.

Originality and accuracy?—As far as we know we were first to: unambiguously define $\xi(T)$ physically; recognise the X-ray spectrum problem as a Fredholm equation and prove it ill-posed; use condition number analysis as a measure of maximum useful resolution in astrophysics. Fortunately others (for example ref. 7) do not share Parkinson's view that we have "added nothing new" and our analysis has indeed been used in Skylab data reduction⁸ and in Spacelab proposals. As to our alleged inaccuracy, Parkinson's opening example seems to be of semantic origin for in this context "high resolution" evidently means 10^4 – 10^5 whereas by his second paragraph "high resolution" has been reduced to $\sim 10^3$. Though for $\xi(T)$ analysis

even spectral resolutions greater than 10^4 – 10^5 are "high", spectrometers of $\lambda/\Delta\lambda < 10^5$ were in fact in orbit⁹ as early as 1967.

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Night-time reception of solar radio events

RIIHMAA has reported night-time reception of a solar radio event¹. The observation, in the frequency range 20.85–23.20 MHz, was made at Kiiminki (65°05'N, 25°54'E) on 30 March 1976 with the Sun (and Jupiter) well below the horizon. Night-time reception of solar bursts had also been reported by Smith *et al.*². Such night-time solar radio burst reception might be brought about by anomalous propagation associated with plasma waves in the lower ionospheric E layer. I briefly discuss this phenomenon here and point out the possible importance of large ionospheric electric fields.

Riihmaa¹ argues that "in the present case the bursts must have been guided from the sunlit hemisphere over the polar regions. It may be significant that on the night of 30 March an auroral substorm and a strong blanketing sporadic-E (E_s) were observed. A suitable duct may have existed between the ground and the E_s layer." The total attenuation was estimated to be of the order of ≈ 10 dB (ref. 1 and personal communication).

The question arises as to what mechanism(s) may bring about the anomalous propagation of the bursts into the night-time hemisphere. Since ray bending and geometrical optics considerations do not seem to suffice, one may resort to a slightly more sophisticated approach.

An E layer in which electric fields above a nominal threshold of ~ 25 mV m⁻¹ excite Farley–Buneman waves^{3,4} of sufficiently large amplitude, may provide enough reflectivity to the ≈ 20 MHz radiation and 'guide' it over the polar region into the night-time hemisphere. I have considered the reflectivity of a Farley–Buneman unstable E layer in a brief paper on riometers⁵. For details see ref. 5 and refs therein.

In the present connection we need only note the following. Reflection of 20-MHz radiation (wavelength $\lambda = 15$ m) by a system of plane parallel plasma waves occurs by Bragg scattering when the relation $\lambda = 2\lambda_n \cos\theta$ is satisfied, λ_n being the relevant wavelength of the plasma waves and θ the angle of incidence. With $\alpha = \pi/2 - \theta \ll 1$ the above relation reads $\lambda \approx 2\lambda_n \sin\alpha \approx 2\lambda_n \alpha$. A typical angle α under which 20-MHz 'rays' from the Sun 'graze' from below the bottom part of the polar cap E layer and are reflected into the night-time hemisphere is $\alpha \approx 0.2$ rad $\sim 10^\circ$. Then the appropriate plasma wavelength for reflection is $\lambda_n \approx 40$ m. Plasma wavelengths of ~ 40 m (in the present case the geometry is such that λ_n is to be understood as the plasma wavelength along the geomagnetic field lines) are available among the Farley–Buneman waves generated by large electric fields in the E layer (ref. 5 and refs therein).

To compute the efficiency of reflection is a difficult task, but some estimates can be made. If we use equation (1) of ref. 5 for the (power) reflectivity R , a plasma frequency $f_p = \omega_p/2\pi \approx 4$ MHz, a percentage plasma density modulation $|\Delta n/n| \sim 0.1$, and a number of scatterers along the ray path $N \sim 10^4$ m per

40 m $\sim 2.5 \times 10^2$, we find $R \approx 6\%$, that is a reflectivity high enough for this reflection process to be of possible significance in 'guiding' the 20-MHz radiation into the high latitude night-side. Note that only one or at most two reflections from the ionosphere are required.

It seems of some relevance that, at the time of Riihmaa's observation, the K_p index was 4⁻, indicative of polar cap electric fields above the Farley–Buneman threshold. The possibility arises of testing the proposed mechanism by determining whether night-time reception of solar radio bursts occurs preferentially when large ionospheric electric fields exist over the polar cap. Were a correlation to be found, it would be in line with the observed correlation between high intensity polar cap electric fields and the occurrence of the Slant E Condition (SEC) in high latitude ionograms^{6,7}.

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Why is the Maier–Saupe theory of nematic liquid crystals so successful?

THE Maier–Saupe theory of nematic liquid crystals is founded on a molecular field treatment of long-range contributions to the intermolecular potential and ignores the important short-range forces. Nonetheless this theory is particularly successful in predicting the orientational properties of real nematics. Here we propose a model which accounts both for the success of the Maier–Saupe theory and the failure of theories based exclusively on short-range forces.

The liquid crystal phase of a nematogen is characterised by the long-range orientational order which is destroyed at the transition to the isotropic fluid. The Maier–Saupe theory has been particularly successful in accounting, not only for the order–disorder transition, but also for the orientational properties of the nematic mesophase¹. The theory is founded on the molecular field approximation applied to a weak anisotropic pair potential; this then gives the effective orientational potential for a single particle as

$$U(\cos\theta) = \bar{u}_2 \bar{P}_2 P_2(\cos\theta)$$

where θ is the angle between the molecular symmetry axis and the director. The order parameter, $\bar{P}_2$, is the ensemble average of the second Legendre polynomial and $\bar{u}_2$ is a combination of the averaged anisotropic interaction parameters. Because the theory contains just one unknown, $\bar{u}_2$, the change at the transition in the order parameter ($\bar{P}_2^{(K)}$) and the entropy ($\Delta S^{(K)}$) are predicted to be universal properties of the nematogen. This prediction is in reasonable agreement with experiment; in addition the calculated values for $\bar{P}_2^{(K)}$ of 0.43 and $\Delta S^{(K)}/R$ of 0.417 are found to be in quite good accord with the observed values^{2,3}. The agreement with experiment can be made perfect by extending the Maier–Saupe theory in a variety of ways. These include the addition of terms to the intermolecular potential to allow for higher rank interactions⁴ and deviations from molecular cylindrical symmetry⁵ as well as by taking the molecular field approximation to higher order⁶.

We shall not consider these extensions, however, because our aim is to understand the reasons for the success of the Maier-Saupe theory rather than to improve it further. In particular we shall consider whether its achievements are accidental or whether they provide an insight into the role of short- and long-range forces in establishing nematic order. Despite its qualitative and even semi-quantitative success the Maier-Saupe theory has been widely criticised (refs 7, 8 and M. A. Cotter, in preparation). One of the major criticisms stems from the foundation of the theory on an anisotropic pair potential which is apparently determined solely by London dispersion forces⁷. As a consequence the nematic-isotropic transition temperature for an ensemble of rod-like particles is predicted to be^{1,4}

$$T_K = 4.541 \times 3h\nu\Delta\alpha^2/20k r^6$$

It is possible therefore to test the theory by attempting to calculate the transition temperature from a knowledge of the anisotropy, $\Delta\alpha$, in the molecular polarisability tensor. Thus order of magnitude estimates of $\Delta\alpha$ and the dispersive energy are found to yield transition temperatures which are considerably lower than those observed^{7,8}. Of course these estimates are themselves open to criticism because the internal electric field in an anisotropic medium is not well understood⁹. None-the-less it is doubtful if dispersion forces, as envisaged by Maier and Saupe, could be entirely responsible for the existence of liquid crystals.

A second attack on the theory is concerned with its complete neglect of anisotropic short-range repulsive forces which should be of special importance for the elongated molecules forming nematogens. This neglect would seem to be quite unjustified as it is now widely accepted that scalar repulsive forces have a dominant role in determining the organisation in simple fluids¹⁰. Such considerations have prompted the development of theories based on purely short-range anisotropic forces using a variety of approximations, which include the Onsager approach¹¹, lattice summations¹² and scaled particle theory¹³. In every case the order parameter and entropy change at the predicted phase transition were found to be much larger than their experimental counterparts. For example, the Onsager theory¹¹ gives the order parameter $\bar{P}_2^{(K)}$ at the transition as 0.85 which is over twice the value normally observed for nematics while the entropy change $\Delta S^{(K)}/R$ is predicted to be 8 which is in marked contrast to the observed values of about 0.3 (refs 2, 3). Of course the high length-to-breadth ratio of the constituent particles in the systems discussed by Onsager, are not realistic for a nematic, however the scaled particle theories do not suffer from this fault, and in addition, have been reasonably successful when applied to simple fluids¹⁰. None-the-less the predicted values of $\bar{P}_2^{(K)}$ are still found to be in excess of those determined by experiment, similarly the discontinuity in the density at the order-disorder transition is also predicted to be too large¹³. Such failings do not seem to be a consequence of the necessary approximations inherent in the theories for a computer-simulation experiment based on a continuous repulsive potential also yields an unrealistically high value for the order parameter¹⁴.

The theories based on interactions between hard particles suffer from another weakness, namely the absence of any direct dependence of the orientational properties on temperature. Thus, even though it may be reasonable to suppose that the order-disorder transition depends solely on density for a suspension of macromolecules, it is difficult to believe that density alone determines the transition in thermotropic liquid crystals. Indeed this failure is highlighted by the inability of these theories to account for the observed temperature dependence of the order parameter, $\bar{P}_2$, at constant volume¹⁵. In marked contrast the Maier-Saupe theory is able to provide a temperature dependence in very good agreement with experiment¹⁶.

We are faced, therefore, with an apparent dilemma. On the one hand theories based on short-range repulsive forces, which are expected to make the dominant contribution to the pair

potential, yield results in poor accord with experiment. While on the other hand, the Maier-Saupe theory, founded on long-range dispersion forces, provides an excellent description of the orientational properties of real nematogens. A formal solution to this dilemma is provided by the molecular field theories based on a completely general form of the total intermolecular potential⁴. This is separated into a scalar component and an anisotropic part¹⁷ which is then written in terms of the Pople expansion. The orientational part of the single particle potential is found to be a series whose first term has the same form as the Maier-Saupe result, although the coefficient $\bar{u}_2$ now contains, in principle, contributions from all anisotropic forces. The rapid convergence of the expansion for the single-particle potential is guaranteed, at least for nematics, by the marked decrease of the general order parameter, $\bar{P}_L$, with its rank L . Consequently, it is not necessary for the Pople expansion itself to converge rapidly; indeed, for small intermolecular separations the convergence is slow. The Maier-Saupe theory is, therefore, equivalent in form to one which includes both long- and short-range contributions to the anisotropic intermolecular potential.

There are, however, several unsatisfactory features of this analysis. In the first instance because all intermolecular forces contribute to $\bar{u}_2$ this is virtually impossible to determine and so the theory cannot be tested by calculating the nematic-isotropic transition temperature. By the same token, we are unable to discern which anisotropic forces are the most important for the formation of nematic liquid crystals. Finally, the general theory shows that the distance dependence of the anisotropic potential must be averaged over the pair distribution function appropriate for particles interacting with a purely scalar potential¹⁷. Such an averaging procedure seems physically unrealistic and so quite different schemes have been devised which allow for the anisotropy of the short-range forces.

Here we propose a solution to these difficulties. We suggest that both short- and long-range forces are important in determining the molecular organisation in a nematic phase but that they operate at quite different levels. In our model we suppose that the short-range forces are responsible for the formation of highly ordered groups or clusters of molecules. These forces may be repulsive as suggested by the behaviour of simple fluids, although with molecules as complex as those in nematogens specific anisotropic electronic interactions could also be involved. Physically we might imagine the cluster to be less anisometric than its constituent particles; as a consequence the anisotropic short-range forces between clusters would become of minor importance. Secondly, the anisotropy in the polarisability of the cluster will be approximately proportional to the number of particles in the cluster and so the dispersive interaction energy between clusters would become dominant. The net effect of the existence of clusters would be to reduce the anisotropy associated with the short-range forces while increasing the influence of long-range forces. The possibility of cluster formation was mentioned in the original Maier-Saupe theory¹ although its purpose seems to have been to force agreement between the predicted and observed entropy of transition. The other important ingredient of our model is the assumption that the clusters are not destroyed at the nematic-isotropic phase transition. The anisotropic forces between clusters are therefore responsible for the orientational properties of the nematic mesophase. The parameter $\bar{u}_2$ in the effective potential is not then determined by the interaction of two molecules, but by two clusters. Consequently, the coefficient $\bar{u}_2$ will be essentially proportional to the square of the number of particles in a cluster and so, even if dispersion forces did dominate the long-range interactions, it would still be possible to produce the necessary increase in the predicted transition temperature by the inclusion of a relatively small number of particles in a cluster. We can also understand the failure of theories based on repulsive forces. The fundamental unit in these calculations is a single particle and at the transition the orientational order is completely destroyed which produces a large increase in entropy. Whereas for the cluster model the transition unlocks the

ordering of the clusters but not that of the molecules within the cluster; consequently, the entropy of transition is small.

The notion of cluster formation in nematogens is apparently of value. It is important, however, to appreciate exactly what is meant by a cluster; indeed, such a concept has already been questioned because it was thought that the cluster should have some physical existence, as an independent object⁸. This existence is not necessary. In the language of statistical mechanics the cluster would be described in terms of the spatial and orientational pair distribution function. Then our model is equivalent to the assumption that the distribution function exhibits short-range order, over several molecular distances, and this order is not destroyed at the nematic-isotropic transition. Consequently, the variation in the orientational properties on passing from one phase to the other will be determined by the long-range intermolecular forces corresponding to distances over which the distribution function does change.

Finally, we must see if there is any other evidence, apart from the success of the Maier-Saupe theory and the failure of hard particle treatments, with which to support our model. Clearly, X-ray and neutron scattering studies are of particular significance because the scattering pattern is directly related to the distribution functions. Such experiments have been performed and they do support the cluster model. For example, the average intermolecular spacing is found from X-ray studies to be about 5.0 Å; this spacing exhibits a weak temperature dependence and is continuous at the nematic-isotropic transition¹⁸. In addition, the high local molecular order is also found to extend for about five molecular diameters but to be insensitive to temperature even at the transition. Preliminary investigations of 4,4'-dimethoxyazobenzene based on coherent neutron scattering also indicate the existence of high local ordering present in both nematic and isotropic phases but with the molecular planes stacked parallel¹⁹. There is additional, although less direct evidence for the existence and preservation of clusters above and below the phase transition. Thus the far-infrared or Poley absorption of 4-methoxybenzylidene-4'-*n*-butylaniline exhibits only a minor change in frequency as the temperature is varied through the order-disorder transition²⁰. Since the absorption frequency is determined by the short-range contributions to the intermolecular potential, these results imply that the short-range part of the distribution function does not change when the ordered phase is destroyed.

In conclusion, we assert that both short and long-range forces are important in determining the molecular organisation in a nematic liquid crystal. But, because the short-range part of the pair distribution function does not change appreciably at the nematic-isotropic transition, it is the long-range contribution to the pair potential which determines this transition. Consequently, the Maier-Saupe theory, founded on such forces, provides a good description of nematic liquid crystals, whereas theories based on short-range forces are invariably less successful.

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New shock effects in the phosphide and carbide phases of Cañon Diablo iron meteorites

WE report a hitherto unrecorded variety of shock-induced damage in the cohenite, (FeNi)₃C, of a 'rim type' specimen of Cañon Diablo. The essential feature of the new effect is the wedge-shaped area of cloudy appearance that penetrates into the cohenite from the original cohenite-kamacite interface (see Fig. 1). The figure also shows the reheated, α_2 , condition of the kamacite and the dark-etching zone of carbon diffusion at the cohenite-kamacite interface; effects that have often been reported in 'rim type' samples of Cañon Diablo¹⁻³ and have been produced artificially by shock¹ or isothermal reheating⁴.



Fig. 1 Etched 1% Nital. Dark etching wedge of cloudy material penetrates structureless white cohenite. α_2 matrix, white, granular. Black carbon diffusion border at cohenite- α_2 interface. Note small unaltered phosphide on outer elbow of cohenite near centre of field. Field of view 0.63×0.42 mm.

Figure 2 represents the same feature at higher magnification and shows a characteristic pattern of swirls within the cloudy area.

Figure 3 is a scanning electron micrograph which demonstrates the duplex condition of the cloudy area. Microprobe point analyses of the light background in Fig. 3 were erratic but generally similar to unaltered cohenite, whereas point analyses of the dark inclusions consistently gave slightly higher nickel values. No phosphorus was detected within the cloudy area.

These observations were made on polished and etched sections of a 147-g specimen (number H 37.546 purchased from the American Meteorite Laboratory) and within this specimen numerous examples of the effect were encountered. Some of the cloudy areas in the cohenite were associated with incompletely reacted remnants of phosphide, which commonly occurs as small particles at the cohenite-kamacite interface. Larger areas of cloudy material extend deep into the cohenite crystals and sometimes completely transect them. In specimen H 37.546 these larger areas of cloudy material are occasionally accompanied by microscopically visible decomposition of the cohenite to graphite.

Under polarised light the cohenite appears polycrystalline but shows accentuated recrystallisation along the trace of

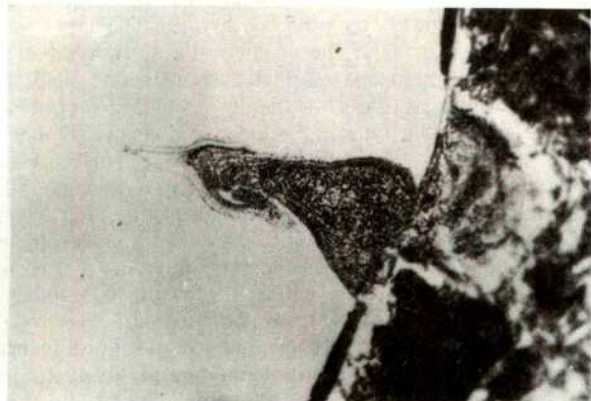


Fig. 2 Detail from Fig. 1 showing swirls within the cloudy wedge. Field of view 0.16×0.10 mm.

shear zones. Visible shear displacements were observed in the cohenite and in taenite. In the bulk kamacite any possible shear traces were obscured by the reheated, α_2 , condition but a small globule of kamacite that was enclosed by cohenite showed a sharply defined shear displacement that lay along a line of enhanced recrystallisation in the surrounding cohenite. Specimen H 37.546 also contained occasional filaments of phosphide located between adjacent kamacite bands. In most instances the only indication of shock reheating shown by this phosphide was the development of minor thorns⁴ at the phosphide-kamacite interface. However, a portion of this phosphide was found to show a fine duplex appearance similar to the cloudy areas observed in the cohenite. Microprobe analysis of this material showed no detectable change of nickel or phosphorus content when there was no detectable extension of the material beyond the original phosphide-kamacite interface. This effect may be called 'in-situ' melting of the phosphide; it appeared to be distributed along shear zones in the specimen and it has probably resulted from the effect of local shear superimposed on a general level of shock that was just insufficient to induce general melting of the phosphide.

The combination of shear, shock and reheating effects observed in this specimen are consistent with the following interpretation of the wedges of cloudy material observed in cohenite, Figs 1–3. The average or bulk level of shock in this specimen was not sufficient to induce melting of phosphide at the kamacite grain boundaries nor of the smaller phosphide particles at cohenite-kamacite interfaces. However, local shear, superimposed on the overall shock, provoked local 'in-situ' melting of phosphide. Some of the small phosphides at the cohenite-kamacite interfaces were

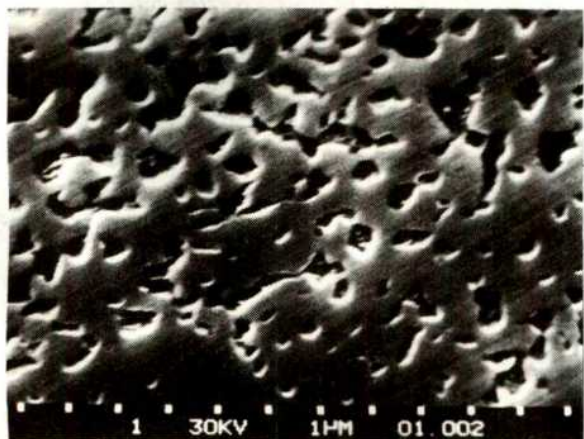


Fig. 3 Scanning electron micrograph of cloudy area in Fig. 2. Field of view 0.015×0.012 mm.

subject to this combination of effects and underwent incipient melting, with the result that the melt penetrated along shear zones in the carbide either as a wedge or as a more extensive band. In either case the process may be visualised as a combination of incipient melting, together with melt penetration along a zone of internal brecciation in the brittle cohenite. As judged by the extent of penetration this would result in considerable ($>10^{-3}$) dilution of the nickel and phosphorus content of the original phosphide. After these shock events the sample reverted to a residual temperature that was sufficiently high to allow some annealing of the earlier shock structure, as is shown by the visible graphitisation in the cohenite.

Following these observations on specimen H 37.546 we re-examined the 'rim' specimens of Axon and Couper² and found cloudy wedges as rare minor features in the cohenite of their specimens 34.5421; 34.2731 and 34.2737. However, the effect was pronounced in specimen 34.FRI. This latter specimen contained major shear displacements and also considerable quantities of shock melted sulphide which confused the identification of the wedge effect. Fortunately, sulphide is not present in the new specimen H 37.546.

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A possible effect of ice ages on the Earth's magnetic field

ICE ages and reversals of the Earth's magnetic field are two widely different geophysical phenomena; but they may be casually linked by the following mechanism. The moment of inertia of the Earth will be changed by variations in size of polar ice sheets and the resulting redistribution of water mass. To conserve angular momentum the Earth's rotation must therefore change. Calculations on the basis of a simple model then suggest that conditions at the core-mantle boundary may be perturbed in such a way as to affect the generation of the Earth's magnetic field.

Although it is difficult to confirm a connection between climatic and geomagnetic changes, such a relationship could have important consequences in several fields. For example, the relatively well dated palaeomagnetic chronology could be used to increase the resolution of identifying and dating palaeoclimatic episodes; and modelling ways in which the reversal frequency of the Earth's magnetic field might change, or modelling even the mechanism of a field reversal itself, could be constrained by a need to be compatible with changes occurring from variations in the Earth's rotation. Also, this may be a mechanism for using solar energy to drive core motions.

A full analysis of the effect of variations in global ice volume and distribution on the Earth's speed of rotation would require a spherical harmonic approach. Although the formalism exists¹ the calculations do not yet appear in the literature. For simplicity, therefore, estimates of the order of magnitude of quantities involved are found from an idealised model to test if the hypothesis is worth further consideration. A rigid mantle is assumed to be decoupled from the core but initially rotating at the same rate. The atmosphere and ocean are assumed to

rotate with the mantle. The difference in inertia between a uniform layer of water, equivalent to a thin spherical shell, and a polar ice cap composed of the same mass of water circling the globe as an annulus is given by

$$\Delta I = I_s - I_i$$

where $I_s = (\frac{2}{3})Mr^2$ and $I_i = M(r \cos \phi)^2$, M is the mass of water or ice, r is the radius of the Earth and ϕ is the mean latitude of the ice sheet. Assuming that the angular momentum stays constant, then the change in rotational energy is given by $\frac{1}{2}\Delta I\omega^2$ where ω is the angular velocity of the Earth. Taking M as 4.3×10^{19} kg (equivalent to a sea level change of about 100 m), r as 6.4×10^6 m, ϕ as 60° and ω as 7.27×10^{-5} rad s⁻¹ then the change in rotational energy is 1.9×10^{24} J. The fractional change in the angular velocity, the length of the day and the moment of inertia is 10^{-5} , in agreement with other estimates². Because in the model the core is decoupled from the mantle there will be a relative velocity of around 2×10^{-3} m s⁻¹ at the core-mantle boundary. Complete coupling between core and mantle will change the core energy by about 1.9×10^{23} J, taking the moment of inertia of the core to be a tenth that of the mantle.

Before exploring the implication of these calculations, the effect of isostasy and changes in the Earth's gravitational potential will be discussed. The timescale for ice sheet growth or decay³ and the isostatic response time⁴ are often considered to be of the same order of magnitude, around 2,000 yr. Therefore if the growth or decay of an ice sheet is such that there is a linear change in the moment of inertia over a time T , and if isostatic adjustment occurs as an exponential change of the moment of inertia with a response time equal to T , then the maximum change in the moment of inertia is reduced by e^{-1} . The fractional changes in the length of the day and the speed of rotation of the Earth will be affected in a similar way. Although not contained in the model considered here, it is important to realise that even when an ice sheet has reached isostatic equilibrium there is still a small difference in the moment of inertia compared with the case where there is no ice sheet⁵; previous calculations of the effects of fluctuating ice volumes on the rotation of the Earth have usually assumed full isostatic compensation⁶ and have thus ignored the much larger transient changes. Farrell and Clark¹ have shown that the gravitational attraction of an ice sheet prevents eustatic sea level changes occurring as a uniform layer of water because the resulting ocean surface would not be an equipotential (that is, there is not a constant change in sea level worldwide). The effect is greatest near the ice sheet and only slightly increases the change in the moment of inertia previously calculated. Changes in gravitational and elastic energy are henceforth ignored.

Assuming a timescale of around 2,000 yr for variations in the size of ice sheets and for isostatic relaxation, an upper limit for the rate of average energy dissipation in the core would be 5×10^{12} W. Estimates⁶ of the power requirements for the geodynamo range from 10^9 to 10^{12} W. Therefore a perturbation of the magnetic field cannot be immediately discounted on energetic grounds. A possible core-mantle coupling mechanism for transferring energy to the core is one suggested by Hide⁷ to explain the decade variations in the length of the day, which are similar in magnitude to those arising here. Shallow topographic features at the core-mantle interface allow horizontal stresses to transfer angular momentum between core and mantle, whereas viscous and electromagnetic torques are not strong enough. Supposing that the polarity of the field depends on comparatively minor features of the pattern of core motions, Hide⁷ has suggested that changes in topography or the temperature field on the core-mantle boundary can lead to changes in the frequency of field reversals. The westward drift of the Earth's non-dipole field at around 3×10^{-4} m s⁻¹ has been thought to indicate the magnitude of core motions⁷. In the model considered here, this velocity would be reached after 300 yr. Timescales of this order seem to be present in some

features of the Earth's non-dipole magnetic field⁸. Precessional forces are an alternative coupling mechanism. They are thought to be insufficient to drive the geomagnetic dynamo⁹, but may still be large enough to modulate the magnetic field. Precessional torques at the core-mantle boundary would arise from changes in the mantle's principal moments of inertia⁹. Theoretical studies of dynamos generated by motions of a conducting fluid have shown their sensitivity to their boundary conditions⁹. It is therefore plausible that a continuous change in the speed of rotation of the Earth over a sufficiently long time could alter conditions at the core-mantle boundary and perturb the magnetic field or even cause a reversal. A number of mathematical models¹⁰⁻¹³ developed to explain the observed frequency distribution of polarity intervals all require reversals to occur by random variations in the orientation or distribution of subsidiary dipoles. It is therefore implied that the energy needed to trigger a field reversal could be much less than that needed to sustain dynamo action.

Evidence for a correlation between glacial periods and changes in the magnetic field is hard to evaluate because of the difficulty in accurately identifying and dating variations in both parameters. It is interesting though to speculate on a number of observed coincidences between changes in the magnetic field and in various other phenomena which might be explained by the hypothesis presented here. Wollin *et al.*¹⁴ have found from an examination of some deep-sea sedimentary cores that changes in intensity of the magnetic field could be correlated over the last 2 Myr with climatic changes represented both by variations in the population of foraminifera and by oxygen isotope changes thought to indicate fluctuations in ice volume. Kent and Opdyke¹⁵ have also found from spectral analysis of deep-sea sedimentary cores an apparent correlation between variations in intensity of the magnetic field and changes in the earth's obliquity. They suggest that the precessional force on the core will vary with the obliquity, and that their results reflect the modulation of a precessionally-driven dynamo by changes in the obliquity. Climatic changes affecting the precession of the earth could thus give rise to geomagnetic reversals. Hays¹⁶ has shown that past extinctions of faunal species have occurred nearly simultaneously with reversals of the magnetic field; climatic change associated with glacial activity could increase the environmental stress on the species leading to their destruction. Cox¹⁷ has shown that the reversal frequency of the magnetic field increased about 75 Myr ago and again between 50 and 35 Myr ago. The South Pole has been positioned over the Antarctic continent for about the last 80 Myr (ref. 18), Australasia parted from Antarctica about 50 Myr ago and the Drake Passage joining Antarctica to South America opened about 30 Myr ago¹⁹. This growing thermal isolation of Antarctica led to increasing glacial activity in the Southern Hemisphere²⁰. Harrison and Ramirez²¹ modelled the apparently limited extent of the Laschamp event by combining an axial geocentric dipole with another dipole near the core-mantle boundary beneath Europe; deglaciation of Northern Europe was contemporaneous with this magnetic event, and may be causally associated with it by affecting conditions at the core-mantle boundary. It may be significant that the time taken for the field to complete a reversal is of the order of a few thousand years, the same as the transient non-equilibrium behaviour of the earth at the beginning and end of ice ages.

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The major stratospheric warming of 1976-77

MANY mid-winter warmings of the polar stratosphere have been observed in the last 25 years by means of radiosonde and rocket soundings and, recently, by satellites¹. Major warmings involve a reversal of the normal winter temperature gradient and zonal wind component in the stratosphere between middle and high latitudes. (In normal winter conditions temperatures decrease polewards and winds are westerly). We report here on the behaviour of the temperature and wind fields observed at various levels during a major warming and the indication that, in this case, similar changes occurred in both the troposphere and the stratosphere.

Fig. 1 Mean temperature ($\bar{T}$) over the cap north of 50°N plotted at two-day intervals. Climatological values of monthly mean temperatures, plotted at the mid-point of each month were derived from various publications: +, Crutcher⁴; ▲, Hanevskaja⁵; ■, Labitzke *et al.*⁶.

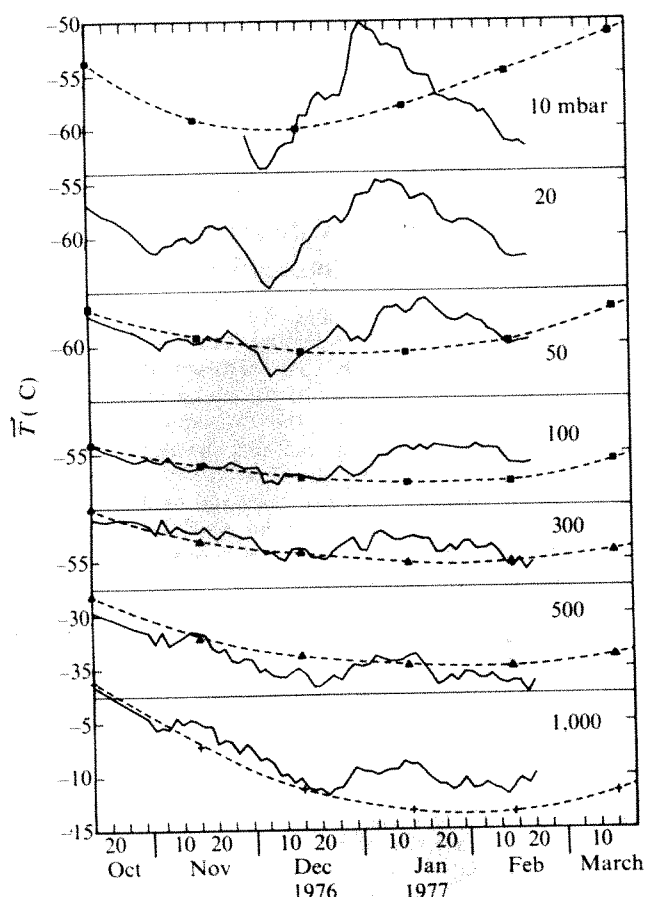


Fig. 2 1,000-500 mbar thickness anomalies (from the 1951-1970 average) for pentad 3, 1977 (11-15 January). Values are shown in geopotential decametres. ●, Grid points at which the anomaly is at least 4σ .

A stratospheric warming which began in December 1976 developed into a major event in January 1977, with zonal mean values of both temperature and geopotential height increasing polewards from 60°N at the 10 and 20 mbar levels. A full analysis of the energetics involved is not yet available (Labitzke *et al.*² have given a synoptic description of the event), but certain unusual aspects of atmospheric behaviour have become apparent from a study of objective analyses for the period.

The Meteorological Office produces twice-daily objective analyses of geopotential height fields at standard pressure levels for the Northern Hemisphere north of about 15°N and, from these analyses, fields of temperature and wind are inferred. We have processed the 00 GMT analyses on alternate days from late 1976, at seven levels (1,000, 500, 300, 100, 50, 20 and 10 mbar) to obtain, at each level, areal mean values of temperature and zonal wind component over the cap north of 50°N.

Variations of temperature during the present winter may be compared with climatological values (Fig. 1). Differences in the treatment of radiosonde data by individual organisations make it difficult to compare absolute values from different sources so the climatological values are included only to demonstrate the average shape of the temperature curves, with the tropospheric minimum occurring in late January or early February. The earlier climatological minimum at 10 mbar probably results from the occurrence, during the five-year period from which the stratospheric mean values were produced, of warmings which affected temperatures from late December onwards.

Two stratospheric warming periods are evident in Fig. 1, the first beginning in early November (during which derived temperatures were not available at 10 mbar) and the second beginning on 6 December and reaching a maximum around 3 January at 10 mbar. During the course of this second stratospheric disturbance, tropospheric temperatures also rose between mid-December and mid-January by some 2 to 3°C. The anomalies of 1,000-500 mbar thickness from the 1951-70 average for the pentad 11-15 January 1977 show (Fig. 2) predominantly positive values north of 50°N. This chart contains five grid points with anomalies of 4σ

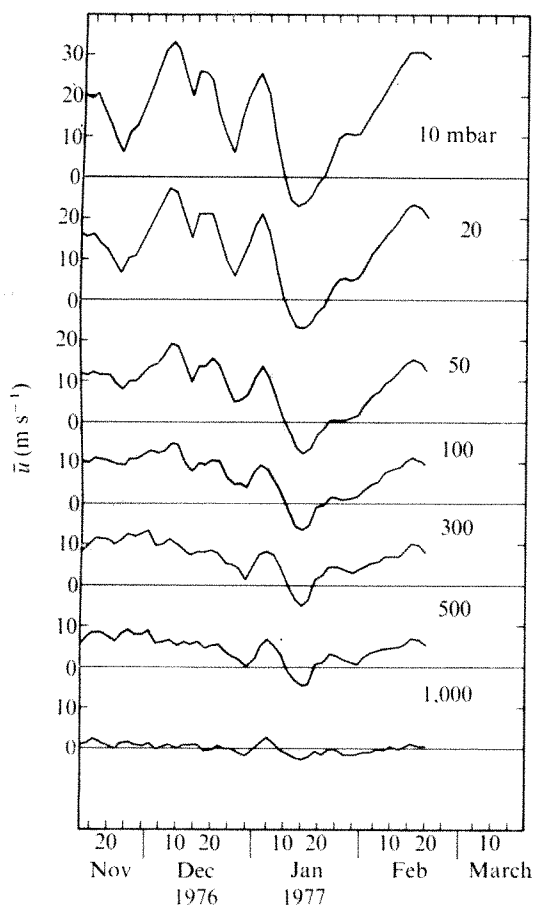


Fig. 3 Mean zonal wind component ($\bar{u}$) over the cap north of 50°N plotted at two-day intervals, (westerly winds are positive). Allowance has been made for surface friction in calculating the winds at 1,000 mbar.

or more where σ denotes the standard deviation for that grid point derived from the 20 values for the corresponding pentad in the years 1951–70; they are all positive and are centred at 80°N , 90°W . The chart for the following 5-d period contains four grid points having similar anomalies; these are centred at 80°N , 145°E . Examination of corresponding thickness anomalies from 50°N to the pole for the previous six winters revealed that only at 21 grid points (of a possible 29,232) did the anomaly reach 4σ . The occurrence of nine extreme values in one 10-d period of mid-January 1977 is in marked contrast to this recent average of three or four per winter: this uncommon tropospheric warming during a stratospheric warming period represents a situation of obvious interest. On the other hand, the apparent lag between the start of the warming at the upper levels on 6 December and the warming in the lower troposphere (about 22 December) should not be interpreted as an indication that the tropospheric warming is caused by the stratospheric disturbance; the determination of any causal relationship between the warmings must await a full analysis of the energetics involved.

The mean zonal component north of 50°N ($\bar{u}$) 10 mbar reversed between 9 and 10 January (Fig. 3). This was associated with a reversal of the meridional gradient of zonal mean temperature in the stratosphere. However, the magnitude of the reversed temperature gradient and the corresponding rate of decrease of $\bar{u}$ with height were small at this stage (and indeed remained so in comparison with a previous major warming in 1967–68 (ref. 2)). The 10-mbar wind reversal was achieved largely because $\bar{u}$ in

the troposphere had also decreased; by 11 January easterlies were present at all levels.

The vertical continuity, so evident during the easterly period, was, in fact, present from late December onwards. A degree of continuity was also present during the minor warming in November minimum values of $\bar{u}$ (though still positive) occurred at all levels at approximately the same time (22–24 November). These results are consistent with the findings of Julian and Labitzke³ who provided evidence for an association between stratospheric warming and a reduction of the 500 mbar zonal wind component in the region 50°N – 70°N (used as an indicator of ‘blocking’). It may well be that the atmospheric behaviour observed during December and January represents an extreme example of this association.

The synoptic situation associated with the anomalously high polar temperatures and easterly winds of 15 January was dominated by a geopotential high near the North Pole at all levels from the surface up to at least 10 mbar. Tropospheric charts for the easterly period thus have much of the appearance of the typical stratospheric charts during warmings (though also containing features of higher wave numbers not present in the stratosphere). In view of this similarity between these two atmospheric layers it may be unjustifiably restrictive to describe the events as only a stratospheric warming.

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Zonal winds associated with the dry period 1975–76 in England and Wales

THE low rainfall in England and Wales during the period May 1975 to August 1976 was discussed by Morris and Ratcliffe¹ and was shown to be associated with a progressive shift northwards in the position of the mean jetstream over the north-east Atlantic during the three successive years 1974–76. We report here that marked circulation changes in the 500-mbar (5.5 km) flow during the dry period occurred both in the vicinity of the British Isles and over the central Pacific. The flow is compared with an analysis of 5-d mean charts exhibiting blocking of the 500-mbar flow in the vicinity of the British Isles and it is suggested that the anomalous displacement of the jetstream during the dry period may have been associated with an eastward surge in the subtropical jet off south-east Asia.

The actual position of the maximum wind zones around the northern hemisphere varies from season to season but the mean position is conveniently shown by the mean geostrophic zonal west-east component at 500 mbar for the average period 1951–70 (Fig. 1). A northwards displacement of the jetstream occurs not infrequently when a blocking anticyclone is centred in the vicinity of the British Isles, with the shift usually lasting for 5–15 d. The mean zonal flow was studied on occasions when 5-d mean pressure anomaly

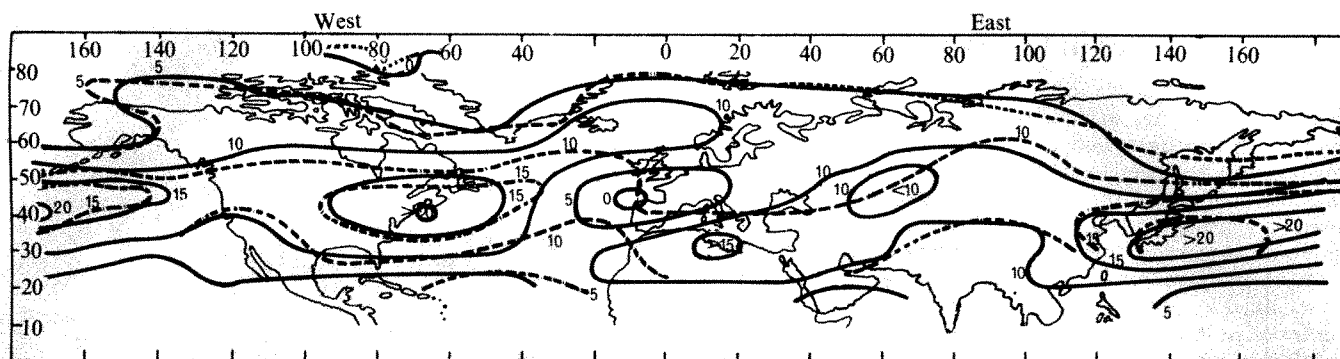


Fig. 1 Broken line, normal (1951-70) 500-mbar zonal components at 5 m s^{-1} intervals. Solid line, composite 500-mbar zonal components at 5 m s^{-1} intervals associated with a positive pressure anomaly in the vicinity of the British Isles.

charts showed a discrete positive pressure anomaly in the vicinity of the British Isles— $50\text{--}60^\circ\text{N}$, $0\text{--}20^\circ\text{W}$ (Fig. 1). On these occasions the maximum in the zonal flow is displaced to the north of the British Isles, further bifurcation of the flow occurs at 60°E , the maximum wind zone off south-east Asia extends further east than the normal position, and there is an eastwards displacement of the south-east Asian trough. Further analysis of the zonal flow shows an almost synchronous eastward surge in the

Pacific temperatures north of 40°N were colder than usual with a mean negative anomaly of approximately 1°C , while temperatures further south were close to normal. Miles³ has also shown that the Pacific was the only sector where the mean north to south temperature gradient in the atmosphere up to 500 mbar (5.5 km) was above average in the latitude band $35\text{--}55^\circ\text{N}$. Sea temperatures are normally conservative over periods of several months and in view of this it is thought that once an anomalous north to south gradient of

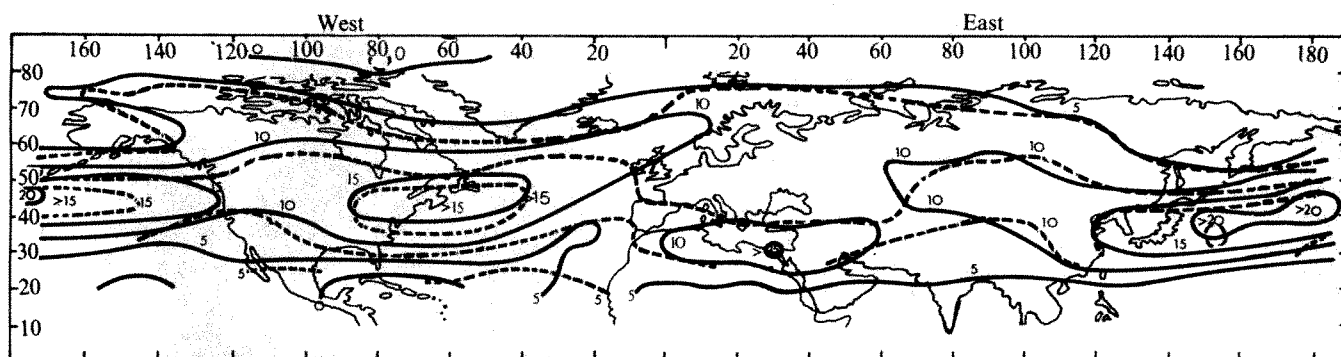


Fig. 2 Solid line, composite 500-mbar zonal components at 5 m s^{-1} intervals for between May 1975 and August 1976. Broken line, pseudo normal (16 months May to August 1951-70) 500-mbar zonal components at 5 m s^{-1} intervals.

500-mbar flow off south-east Asia throughout the year whenever a blocking anticyclone is observed near Britain.

This suggests that the displacement of the jet to the north of the British Isles during the dry period May 1975–August 1976 may have occurred in association with a similar eastwards surge in the subtropical jet off southeast Asia during the same period. Indeed an analysis of the zonal flow at 500 mbar during the dry period in England and Wales which lasted from May 1975 to August 1976 (Fig. 2) shows similar features including extension eastwards of the wind maximum off south-east Asia when compared with the average for the same period. The anomalies in the zonal flow are in the order of plus 4 m s^{-1} both to the north of the British Isles and between 180° and 160°W at 45°N , and are significantly different from the average at the 5% level, using Student's t test. The anomalous eastward displacement of the south-east Asian trough, also observed during the dry period, would by virtue of the hemispherical long wave interdependence result in downstream adjustments within a few days which on the timescales considered here would seem to be synchronous.

During the dry period 1975–76 it has been shown² that

temperature was established in the troposphere, the regime would tend to maintain the enhanced zonal flow over the Pacific and the anomalous eastward position of the south-east Asian trough. It seems, therefore, that the displacement of the jet to the north of the British Isles during the dry period from May 1975 to August 1976 occurred in association with an eastward surge in the sub-tropical jet off south-east Asia during the same period and that the anomalous sea temperature distribution over the Pacific was a factor in maintaining the enhanced flow.

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Weak shock waves in the eruption column

RECENTLY Nairn¹ has made a study of the visible air shock waves which propagate above a volcanic vent in the form of luminous arcs and are closely followed by violent projections of gas and solid ejecta. In ref. 1 it is suggested that this event is caused by a strong explosion within the volcano due to a rapid heating of meteoritic water enclosed in lava pockets. Another type of shock wave formation accompanying volcanic eruption was recorded by one of us (L.D.L.) during an explosive phase of eruption in the Tolbatchik volcanic area (Kamchatka 1975). These waves were smoke and ash which could be observed as optically denser layers rising in the eruption column and probably cannot be attributed to burst phenomena. The present note is based on observations made near the end of the activity of cone II (the middle one in the photographs) when other newly-built pyroclastic cones already showed only weak fumarolic activity.

Over that time (27 August–9 September) the activity of cone II consisted of (1) ejection of a gas stream carrying a great amount of solid particles, (2) jets of ash, lapilli and blocks and (3) a liquid lava emission from the fissure near the cone's foot. Generally the three events took place simultaneously.

The lava temperature near the flow issue is estimated to be about 1,000 °C (ref. 2). The colour of the gas stream at the eruption column base and of blocks and fire jets, taking into account the screen formed by the smoke and ash through which they were observed, suggests they are at temperatures as high as 1,000–1,200 °C. However, the eruption column itself was not luminous and therefore the mean temperature of the gas probably did not exceed 600–400 °C.

Events (1) and (2) were practically continuous but occasional lulls of some 10 min or at most a few hours were recorded. Increases in the violence of gas emission so that even the side of the column exposed to the wind became quite upright—the so-called blastings—were noted also. As a rule the behaviour of the gas stream changed smoothly without any marked preliminary sign but it may be noted that sometimes before the end of a lull in (1) there was an intensification of (2).

In spite of such variations the explosive behaviour might be almost steady for many hours. At these times eruption was accompanied by a roar easily audible within a range of about 10 km and very similar to the noise of a reactive aeroengine fixed in one place. The eruption column was as high as 1 km or more.

At such periods of steady and fairly intense gas ejection one could observe thin optically denser layers cutting across the column. They travelled along the column from the rim of the cone to the cloud with a velocity—relative to the observer—greater than 1 km s⁻¹, most probably of the order of 1.5–3 km s⁻¹. When they met the cloud the layers expanded in diameter and broke up into several parts which in turn rapidly scattered on the lower face of clouds and it appeared to quiver as a result. The visible disintegration of the dense layers was followed by an acoustic shock from above producing in the observer the same sensation as do the air waves generated by aircraft passing through the sound barrier.

The phenomenon described did not occur often, but sometimes several layers followed one another at irregular intervals of some minutes or several tens of minutes.

Emergence of these travelling layers did not correlate with any appreciable changes in the regime of events (1) and/or (2) and was not preceded or followed by distinguishable underground bursts or shots. This behaviour contrasts with the strong atmospheric shock waves discussed in ref. 1: a weak shock wave travelling along the eruption column is unlikely to be connected with explosions within a volcano.

The nature of the explosions in cone II suggests an analogy with a nozzle through which the gas is discharged from the



Fig. 1 *a, b*, Layers of different densities. Photographs showing layers of different densities in the eruption column. Note that the high velocity of layer motion makes their images indistinct. (Photographs by L. D. Livshits).

Earth's interior to the atmosphere. The gas stream may be supersonic. If we assume that the rate of gas flow is sufficiently great that the flow is steady, we have a situation in which dense layers will form. For the estimation of flow rate the Bernoulli equation may be used

$$\frac{1}{2}v_0^2 + C_p T_0 = \frac{1}{2}v^2 + C_p T$$

Suppose that when the gas velocity v_0 is zero the gas temperature T_0 is equal to that of the surrounding rocks (= 1,000–1,200 °C) and that the specific heat $C_p = 1 \text{ J g}^{-1} \text{ K}^{-1}$. We

obtain the result that when cooling to 600–400 °C (the likely temperature of gas in the eruption column) the flow rate may be as great as 1,100 m s⁻¹. For a rough estimate of the sound velocity C in the gas at 600 °C the expression $C \approx (T/T_a)^{1/2}$ gives only about 600 m s⁻¹, where the subscript a refers to the atmospheric conditions. This indicates that the gas flow may indeed be supersonic.

The pressure on the nozzle exit is expressed by the equation

$$p = p_0 \left(1 - \frac{\gamma-1}{2} \frac{v^2}{C_0^2} \right) \frac{\gamma}{(\gamma-1)}$$

where p_0 , C_0 and p , C are the pressure and sound velocity of gas at the nozzle entrance and exit respectively.

If the jet pressure is a positive one ($p > p_a$) the jet stream will expand laterally (in the expansion wave). If the jet pressure is less than atmospheric ($p < p_a$), a pressure discontinuity of the shock type is initiated. In this shock wave (which is always a weak one) the value of the pressure is increased to p_a . To evaluate the gas outlet velocity v corresponding to $p = p_a$ we can use the expression

$$v^* = C_0 \left(\frac{2}{\gamma-1} \right)^{1/2} \left[1 - \left(\frac{p_a}{p_0} \right)^{(\gamma-1)/\gamma} \right]^{1/2} \approx C_0 \left(\frac{2}{\gamma-1} \right)^{1/2}$$

By putting $(p_a/p_0)^{(\gamma-1)/\gamma} \ll 1$, $C_0 \approx C_a(T_0/T)^{1/2}$, $\gamma = 1.4$ we obtain a value of v^* of approximately 1600 m s⁻¹. The value of v^* determines the position of the shock wave in reference to the nozzle exit: when the gas velocity v is greater than v^* the pressure in the channel is reduced and the shock wave moves upstream toward the nozzle entry; when v is less than v^* , the shock wave separates and moves in downstream direction. In 'normal' conditions such a surge would diverge and disintegrate right after separation. But the divergence of the weak shock wave under consideration here is oppressed by acting as a waveguide—the hot gas column is surrounded by cooler atmospheric walls of greater acoustic stiffness (the product of density and velocity of sound). So after separation the shock wave travels along the eruption column with a velocity equal to the velocity of sound in a hot gas. The velocity of the shock wave relative to the observer would be ~ 2 km s⁻¹ as was visually estimated.

In the cloud the acoustic stiffness of the column walls changes and the shock wave is able to diverge. The process is enhanced by reflections of the shock wave especially downwards against the supersonic gas flow. These create a zone of superpressure at the level of lower edge of cloud. The expansion of superpressured gas causes the visible effect of local motion in the lower part of the cloud and generates the sonic bang mentioned above.

The model presented here allows interpretation of phenomenon described in terms of the gas dynamics of the jetstream.

For strong shock waves the column of hot gas and tephra did not serve as a waveguide. Therefore the flashing arcs would pass in the atmosphere irrespective of presence of eruption column (as has been drawn by Perret³) or in its absence, as one can see on photographs in Nairn's paper¹.

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¹ Nairn, I. A. *Nature* **259**, 190–192 (1976).

² Tchirkov, A. M. *Priroda* **7**, 80–93 (1976) (in Russian).

³ Green, J. & Short, N. M. *Volcanic Landforms and Surface Features*, plate 9B, (Springer-Verlag, New York, 1971).

Singlet oxygen in natural waters

MANY studies have shown that singlet molecular oxygen can oxidise a variety of organic substances^{1–7}. These studies have included biologically important compounds present in the aquatic environment such as amino acids^{4,7} and pollutants like polycyclic aromatic hydrocarbons⁵ and pesticides⁶. No data, however, have been obtained to demonstrate that reactions involving singlet oxygen (often called oxygenations) are sufficiently rapid to be significant in natural waters. The most likely mechanism for oxygenation in the environment was originally proposed by Kautsky⁸ by which light energy absorbed by a sensitizer is transferred to ground-state oxygen to form singlet oxygen, which in turn reacts with the organic substance, or 'acceptor' (A), to form a peroxide. Here we present evidence that rapid photochemical generation of singlet oxygen occurs in inland and coastal water bodies of the south-eastern United States.

Samples from selected water bodies were collected in pre-cleaned 1-l bottles with aluminium foil-lined caps. In all cases, water samples were obtained from the top half-metre of the water body and refrigerated at 5 °C before use. Very turbid samples from the Mississippi and a pond in Athens, Georgia, were centrifuged before use to facilitate analysis by liquid chromatography. All other samples were used with no pre-treatment. The samples exhibited a wide range of pH and total organic carbon. (Table 1).

The singlet oxygen, a short-lived species in water⁹, was chemically trapped by 2,5-dimethylfuran (DMF) that was added to the water samples. DMF was chosen because it is highly reactive towards singlet oxygen and its photo-oxygenation products have been well characterised¹⁰. Because DMF has a high tendency to volatilise from water, kinetic studies were conducted in cells equipped with gas-tight Teflon stopcocks. We analysed for DMF and the photoproducts by high-pressure liquid chromatography (HPLC) using 40% methanol–water as the mobile phase and ODS as the stationary phase.

Detailed product studies were conducted on the brown-coloured water from the Okefenokee Swamp. A solution of DMF in air-saturated swamp water was exposed to sunlight for an hour, then the products were collected on prewashed XAD-4 resin¹¹ and isolated by preparative HPLC. The major product (85% yield) was identified as *cis*-1,2-diacetylene by comparison of its mass and NMR spectra with spectra of an authentic sample (mass spectrum: m/e (rel. intensity), 112 (7), 96 (59), 69 (29), 43 (100); NMR (CDCl₃), δ 2.30 (singlet, 6H), δ 6.36 (singlet, 2H)). Two minor products were formed, one of which was identified as *trans*-1,2-diacetylene¹². Foote and his co-workers¹⁰ have shown that DMF reacts with singlet oxygen to form a peroxide which decomposes to diacetylene in water. HPLC analysis indicated that the same products were formed when solutions of DMF in the other water samples were exposed to sunlight. The DMF did not react in dark controls or when exposed to sunlight in distilled water.

To demonstrate further that singlet oxygen was an intermediate in these reactions, the effects of DABCO (1,4-diazabicyclo[2,2,2]octane) and deuterium oxide were studied (Fig. 1). The photo-oxygenations were strongly inhibited by DABCO, an efficient quencher of singlet oxygen¹³. On the other hand, and again consistent with the singlet oxygen mechanism, the reactions were more rapid in air-saturated 1:1 mixtures of deuterium oxide–natural water than in 1:1 mixtures of distilled water–natural water. The acceleration is attributed to an increase in the lifetime of singlet oxygen in the presence of deuterium oxide⁹. DABCO and deuterium oxide affected the reaction in Okefenokee Swamp water in nearly the same way as they affected photo-oxygenation of DMF using rose bengal, a known singlet oxygen sensitizer¹⁴ (Fig. 1).

When the concentration of acceptor is low, it has little in-

Table 1 Kinetic parameters for photochemical generation of singlet oxygen in natural waters from the south-eastern United States

Water body	pH	Total organic carbon (mg l ⁻¹)	F_{366}^*	$(Y_s)_{366}^\dagger$	Sunlight [‡] $t_{1/2}$ (min)	$[O_2^*] \times 10^{13}$ M§
Okefenokee Swamp, Georgia	4.1	24	0.389	0.056	13	22
Aucilla River, Lamont, Florida	6.0	24	0.446	0.026	16	18
Econfina River near Perry, Florida	4.2	41	0.658	0.032	15	19
Fenholloway River, Foley, Florida	7.7	77	0.941	0.014	17	17
Wakulla River near Wakulla Springs, Florida	8.6	6	0.027	0.060	150	2
St Marks River, St Marks, Florida	8.7	9	0.165	0.030	51	6
Gulf of Mexico, Shell Point, Florida	8.5	6	0.050	0.060	100	3
Gulf of Mexico, Live Oak Island, Florida	8.3	6	0.060	0.052	—	—
Puddle in peanut field, Sylvester, Georgia	6.6	12	0.113	0.065	33	10
Mississippi River, Baton Rouge, Louisiana	8.3	14	0.060	0.093	60	5
Hickory Hills Pond, Athens, Georgia	7.2	4	0.041	0.019	—	—

*Fraction of 366-nm light absorbed in 1.00 cm.

†Quantum efficiency for singlet oxygen formation at 366 nm.

‡Midday half life for photo-oxygenation of 2,5-dimethylfuran under December sunlight.

§Steady-state concentration of singlet oxygen.

fluence on the lifetime of singlet oxygen, τ , and the rate expression for disappearance of acceptor is

$$-\frac{d[A]}{dt} = \frac{I_a Y_s}{\beta} [A] \quad (1)$$

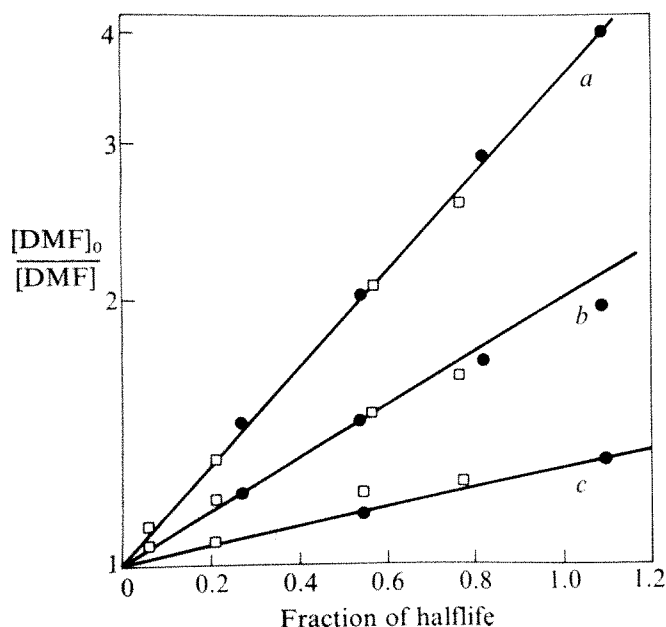
where I_a is the rate of light absorption and Y_s is the quantum efficiency for singlet oxygen generation¹⁵. The constant, β , equals $1/(k_A \tau)$ where k_A is the rate constant for reaction between

singlet oxygen and acceptor and τ is the lifetime of singlet oxygen^{1,9}. Data in Fig. 1 indicate that τ is about the same in distilled and swamp water. To determine values of Y_s for the water samples, air-saturated solutions of 10^{-4} M DMF in natural water and potassium ferrioxalate actinometers¹⁶ were exposed simultaneously to monochromatic light (366 nm) in a 'merry-go-round' apparatus¹⁷. Values of Y_s were computed from slopes of first-order plots, assuming that β for DMF is $1.2 \times 10^{-3} \text{ mol l}^{-1}$ in all the water samples⁹. These values ranged from a low of 0.014 to a high of 0.093 with an average of 0.043 ± 0.019 in our water samples (Table 1). In view of the reported complexity of the substances in natural waters¹⁸, the observed variation in quantum efficiencies was remarkably small.

Of particular environmental interest are the steady-state concentrations of singlet oxygen, $[O_2^*]$, that are photochemically generated by the action of sunlight. Near-surface concentrations of O_2^* were estimated by exposing 1-cm thick solutions of DMF (10^{-4} M) in the natural waters to sunlight in Athens, Georgia, (latitude 34°N) during December. Steady-state concentrations were assumed to equal $0.693 (k_A t_1)^{-1}$, where k_A is $4 \times 10^8 \text{ l mol}^{-1} \text{ s}^{-1}$, the rate constant for the reaction of DMF with singlet oxygen⁹, and t_1 is the first-order half life observed in the sunlight experiments (Table 1). Changes in $[O_2^*]$ were closely paralleled by changes in the rates of O_2^* generation at 366 nm, as reflected by the product, $F_{366} (Y_s)_{366}$ (Table 1). Taking the underwater solar irradiance values reported by Zepp and Cline¹⁹, we estimated that midsummer values are about three times higher than these midwinter values. The highest rates of singlet oxygen formation occurred in water from the highly coloured rivers of north Florida and the Okefenokee Swamp (first four in Table 1). These water bodies contain high concentrations of organics, most of which is fulvic acid, a complex humic substance derived from decayed plants¹⁹. Because most inland and coastal water bodies contain fulvic acid, it is reasonable to infer that photochemical generation of singlet oxygen is a widespread phenomenon in the aquatic environment.

The kinetic parameters in Table 1 can be used to estimate approximate half-lives for photo-oxygenation of dissolved organics in the aquatic environment. Using reported^{4,5} values of k_A , the following half-lives were estimated for photosensitized oxygenation of selected polycyclic aromatics and amino acids in the Okefenokee Swamp water: 7,12-dimethylbenz(a)anthracene, 6 h; naphthacene, 7 h; pentacene, 0.02 h; histidine, 2 h; tryptophan, 2 h; methionine, 3 h. The pesticide, *cis*-resmethrin²⁰, a furan derivative, must react at about the same

Fig. 1 Effect of DABCO and deuterium oxide on photo-oxygenation of 2,5-dimethylfuran (DMF) in Okefenokee Swamp water and in distilled water containing rose bengal; initial DMF concentration $[DMF]_0$, 1.0×10^{-4} M. Duration of light exposure expressed as fraction of half life compared with systems with no added DABCO or deuterium oxide (line *b*). *a*, 1:1 mixture of swamp water or 10^{-4} M aqueous rose bengal with deuterium oxide; *b*, 1:1 mixture of swamp water or 10^{-4} M aqueous rose bengal with distilled water; *c*, 1:1 mixture of swamp water of 10^{-4} M aqueous rose bengal with 0.019 M aqueous DABCO. Light source was a 450-W high-pressure mercury lamp; light was filtered through Corning 7-83 glass filters to isolate 366-nm line. ●, Rose bengal; □ Okefenokee swamp.



rate as DMF. These half lives apply only to organics dissolved in the water. In water containing an appreciable amount of suspended sediments, extremely hydrophobic organics, such as polycyclic aromatics, exist in both the dissolved and sorbed state²¹. Nonetheless, the computed half lives do serve to indicate that singlet oxygen is likely to be an important intermediate in the transformation of certain pollutants and biologically important substances in lakes and rivers. Also, it is possible that the peroxides which form in singlet oxygen reactions may initiate the free radical oxidation of aquatic pollutants.

Finally, these findings suggest that singlet oxygen may be involved in the well known sunlight-induced fading of the colour in natural waters¹⁸. In preliminary studies of this phenomenon, we found that the Okefenokee Swamp water underwent considerable fading when exposed to sunlight for one full day. This fading did not occur when oxygen was removed from the water by degassing before sunlight exposure.

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Low oxygen concentration extends the lifespan of cultured human diploid cells

THE pioneering studies of Hayflick¹ showed that normal human diploid cells of the WI-38 strain have a limited lifespan of approximately 50 ± 10 population doublings (PDL) *in vitro*. It is still not known to what extent this phenomenon is the result of the artificiality of the cell culture system: factors such as nutrition and ambient environmental factors may restrict the ability of these cells to express their true genetic potential. To what extent genetic and environmental factors act to constrain the lifespan of human diploid cells in culture is open to experiment. In the present study we have evaluated one important ambient factor of the environment, the oxygen concentration, on the growth and lifespan of two human diploid cell strains, WI-38 and IMR-90. Our results show that growth at oxygen concentrations less than 20% is enhanced and that an extension of about 25% of the lifespan of both cell types was realised by long term cultivation under 10% oxygen. This indicates that the potential for long term growth in culture of normal diploid cells may

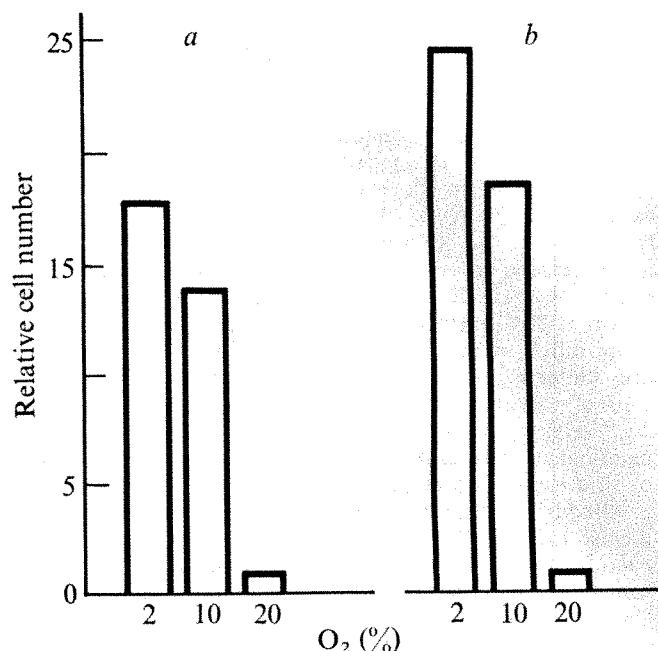


Fig. 1 Effect of oxygen concentration on the growth of IMR-90 (a) and WI-38 (b) human diploid cells. Cells were grown in Eagle's Basal Medium with Hanks' salts (HBME, Pacific Biologicals) to which was added $50 \mu\text{g ml}^{-1}$ aureomycin (Lederle), $2.8 \times 10^{-2} \text{ M}$ N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES, Sigma) and 10% (v/v) non-inactivated foetal calf serum (GIBCO). Young WI-38 cells at PDL 23 and IMR-90 cells at PDL 19 were seeded in T-25 flasks at about 2,000 cells per flask and kept in a desiccator at 37°C at the indicated oxygen concentration for 10 d. Cell counts performed in triplicate for each oxygen concentration are given as relative values based on a value of 1 for the 20% oxygen cell counts: for IMR-90 this was 18×10^3 ; for WI-38 this was 28×10^3 .

be appreciably enhanced by more exact control of environmental factors.

We have studied the effect of oxygen concentration on growth and lifespan, primarily using WI-38 cells which are generally accepted to exhibit a limited *in vitro* lifespan¹. But the normal human lung strain IMR-90 has also received study more recently. Therefore, we felt it important to carry out parallel experiments using both types of cells. Cultures were tested periodically for mycoplasma by the culture method and found to be negative. Cell culture techniques have been described in detail elsewhere^{2,3}. WI-38 and IMR-90 cells were exposed to varying oxygen concentrations in the gas phase over the culture medium, and the effect on growth and lifespan evaluated. The term 'growth' as used in this study is meant as increase in cell number and not as increase in cell size. The population doubling level was used as a rough indication of culture age and is defined as a twofold increase in the number of cells seeded into a vessel².

Individual flasks were seeded with cells in culture medium. For growth studies, cells were seeded at 0.25×10^6 cells per flask and triplicate flasks were counted daily from days 2-7 inclusive of incubation at various oxygen concentrations. For plating experiments, cells were seeded at a low density (2,000 cells per flask) and cells and colonies were counted after 10 d of growth (with no media change) under varying oxygen levels. The flasks were then flushed for 1 min at a flow rate of 1.5 l min^{-1} with various proportions of oxygen in nitrogen with carbon dioxide held constant at 5% at 1 atm and 20°C . The following combinations of gas were used: 2% O₂, 93% N₂, 5% CO₂; 5% O₂, 90% N₂, 5% CO₂; 10% O₂, 85% N₂, 5% CO₂; 20% O₂ (added as 95% dry air which contains 19.99% O₂), 5% CO₂; and 50% O₂, 45% N₂ and 5% CO₂. The gassed flasks were placed in

250-mm desiccators from which the air was removed by vacuum and replaced with the gas mixture used in flushing the flasks.

In short term growth studies, within a given series of subcultures, we have generally observed that concentrations of oxygen below 20%, particularly between 5 and 10%, speed up the growth rate. When the results of many experiments are averaged, however, the results were usually found not to be statistically valid. If cells are seeded at lower density, however, the effects of lowered oxygen concentration on cell growth are apparent, relative to cells grown at 20% oxygen; cells cultivated at 10 and 2% oxygen show much increased growth rates (Fig. 1). Such results are similar to the increased clonal growth rates of cultured cells of various types reported previously^{4,5}, that less than 20% oxygen concentration enhances the growth rate of mammalian cells cultured either attached to the substratum or in a suspension culture (compare with ref. 5).

Although neither WI-38 nor IMR-90 showed reproducibly greater rates of short term growth, it seemed possible that cumulative effects of lower oxygen concentration might be seen in lifespan studies, particularly since cell growth was always inhibited, even in the short term, at oxygen concentrations greater than 20%.

A typical series of experiments showing the effects of 10, 20 and 50% oxygen on the lifespan of WI-38 cells is shown in Fig. 2. At 50% oxygen, the cells phase out after two subcultivations, whereas the control cultures grown at ambient 20% oxygen exhibit a finite lifespan of 56 population doublings. When grown continuously at 10% oxygen, the cells did not phase out until 68 population doublings had elapsed. Table 1 summarises four series of experiments with

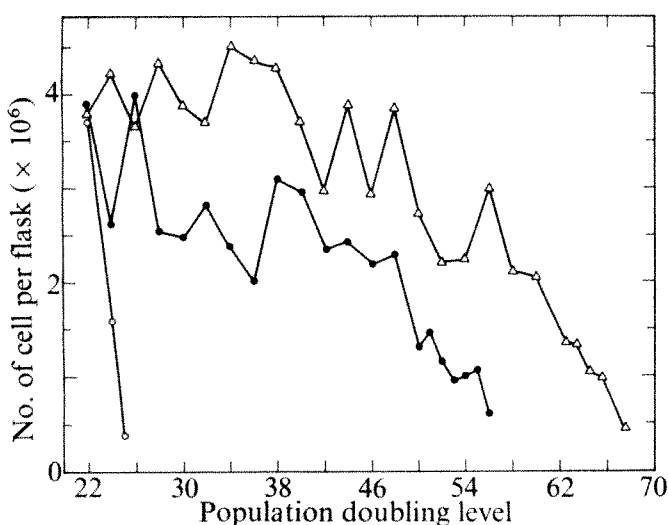


Fig. 2 Effect of oxygen concentration on the lifespan of WI-38 human diploid cells. The parent flask at PDL 18 was subcultivated at about 2.5×10^6 cells per flask in duplicate for each oxygen concentration. Every 7 d the cells were trypsinised, counted, subdivided and regassed. A solution of 0.25% trypsin in Ca- and Mg-free balanced salt solution (Pacific Biologicals) was used to detach cells from flasks for subcultivation. Cell counts were determined using a haemocytometer, or by Coulter Counter Model P64. When necessary, the pH of the culture medium was adjusted by adding 7.5% sodium bicarbonate. PDL was determined by the split ratio used in the subcultivation and is derived from the equation $n = n_0 2^x$ where n = final cell number in a single culture, n_0 = starting cell number, and x = number of doublings². Once each week, cultures were split at ratios of 1:4 for 2×10^6 or more cells, or 1:2 for 1×10^6 to 2×10^6 cells, and the PDL was estimated by adding 2 population doublings at each 1:4 split or 1 PDL at each 1:2 split. If the number of cells was less than 1×10^6 they were resuspended and reseeded. If cells had not reached confluence, they were fed. When cultures would no longer grow into a confluent monolayer of cells within 3 weeks after a 1:2 split following addition of fresh medium each week, the cultures were considered to be at the end of their lifespan. Δ , 10% O_2 ; $\bullet$, 20% O_2 ; $\circ$, 50% O_2 .

Table 1 Effect of oxygen concentration on the lifespan of human diploid cells

	Population doublings*		Increase (%)
	20% O_2	10% O_2	
WI-38			
Series 32	47	65	38
Series 46A	57.5	67.5	17
Series 46B	56.5	67.5	19
Series 46B	57.5	62.5†	9
IMR-90			
Series 5	39	47	21
Series 6	38	50	32

*Started at PDL 20 or 22; values derived from averaging duplicate cell counts.

†Transferred from 20% O_2 to 10% O_2 at PDL 52.5.

WI-38 and two series with IMR-90 with 10% oxygen, showing that cell lifespan is enhanced on average by about 25%. In one case, series 46B for WI-38 where the oxygen concentration was lowered to 10% only after PDL 52.5, the benefit in lifespan extension was only 9%. This suggests that extension of lifespan by lower oxygen may be realised at any stage in the lifespan, and the effect of oxygen concentration on long term growth may be cumulative.

In other studies not reported here (J. Walton collaboration), we have found that cells maintained under 50% oxygen show extensive morphological damage, particularly to mitochondrial and membranous structures. No apparent morphological changes were observed between ambient oxygen and 10% oxygen, except that the morphological changes usually associated with phase III (the senescence phase), were delayed in cells grown at lower oxygen.

The ambient oxygen concentration of 20% normally used in cell cultures is probably higher than that to which cells are exposed *in vivo*. Although cells have evolved several mechanisms for counteracting oxygen toxicity (namely superoxide dismutase, glutathione peroxidase), the possibility remains that cumulative damage arising from O_2 and products of oxidative metabolism may limit the lifespan of cells both *in vivo* and *in vitro*. Previous studies^{4,5} have demonstrated that the growth of cells after low cell density seeding can be enhanced by lowering the oxygen concentration below ambient; in static culture, clonal growth of WI-38 and adult monkey cells was observed by Taylor *et al.*⁴ to be enhanced by lowering the oxygen in the gas phase from ambient to 1%, and that variable growth and reduced plating efficiencies occurred in cultures grown at ambient 20% oxygen. Similar findings were reported by Richter *et al.*⁵ both for neoplastic and non-neoplastic cells from foetal tissues. Our results are consistent with these findings. Furthermore, we have now evaluated the long term influence of lower oxygen concentration on growth of normal human diploid cells in static cultures, and show that an average extension of the lifespan of 25% can be achieved by lowering the oxygen concentration from 20% to 10%.

Oxygen is essential, yet it may be inhibitory for growth if its steady-state concentration is below or above optimal. For two normal human diploid cell strains, 10% O_2 seems to provide optimum conditions for long term growth in culture. In a similar study, Balin *et al.*⁶ reported no extension of WI-38 lifespan by O_2 concentrations between 5.6 and 50 mmHg; the effect of 75 mmHg, equivalent to 10% O_2 , was not investigated; furthermore there was no stimulatory effect with low O_2 , and the same inhibitory effect with high O_2 as we have observed⁷ was found. This suggests that further studies on the effects of oxygen and oxidative damage on cells in culture are important. Other factors which have been reported to extend lifespan of normal human diploid cells (compare refs 3,6-8) such as

hydrocortisone, serum, additional bovine serum albumin and vitamin E, should be evaluated alone and in combination, under lower oxygen concentrations. The extent to which cells can be cultivated *in vitro* in more optimal environmental conditions can thus be determined. The wide use of normal human diploid cells as model systems for ageing studies and virus production, for example, indicates that immense benefits could result if such cells could be cultured on a long term or indefinite basis *in vitro*.

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Mutation and inactivation of mammalian cells by various ionising radiations

THERE is almost no information on the mutagenic effectiveness of the various ionising radiations for man and only limited information about radiations other than X or γ rays for experimental mammals. We have measured inactivation and mutation induction in cultured mammalian cells exposed to various radiations covering a range of linear energy transfer (LET; ref. 1). We show for two cell types that the maximum mutagenic effectiveness of these radiations, relative to X or γ rays, is about twice that for inactivation. Preliminary experiments suggest that specific chromosome aberrations may be associated with mutations induced by radiations of high LET.

LET is a measure of the deposition of energy along the tracks of the charged particles produced in irradiated material. The distribution of energy loss events along a track depends on the type of radiation; events are widely-spaced for radiations of low LET such as X or γ rays, but more closely spaced for radiations of higher LET such as α particles (as from plutonium) and the protons and other secondary ions produced by neutrons in tissue. The effectiveness of a radiation in producing a particular biological response relative to that of X or γ rays is known as its relative biological effectiveness (RBE; see ref. 2 and Table 1 legend). The yield of mutations per unit absorbed dose of radiation in lower organisms and plants is affected by LET (ref. 2). High dose-rate (acute) irradiations of mice have shown that the RBE of fission neutrons for the production of several types of mutation is about 6 in spermatogonia and about 4.5 in maturing oocytes³. RBEs of about 20 for mutation induction have been found after low dose-rate (chronic) irradiation of spermatogonia by fission neutrons³. But neutron irradiations produce tracks of widely differing LETs (for fission neutrons the range is about 10 to over

500 keV μm^{-1}) and the observed RBEs give little indication of the variation of RBE with LET. Compared with whole mammals, cultured mammalian cells offer the advantages of rapidity of assay and ease of irradiation, especially when the radiation is poorly penetrating, and also the opportunity for more detailed analysis of the mechanisms of mutagenesis. Quantitative estimates of mutation induction by low LET radiation can be made for cultured mammalian cells⁴, including freshly-isolated human cells, using a mutation system based on loss of the activity of the purine salvage enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT).

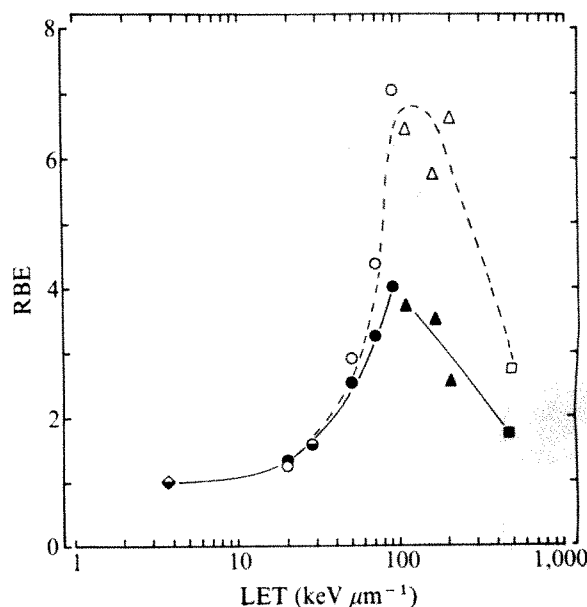
Cultures of freshly-isolated human cells and established V79 hamster cells were irradiated with helium, boron or nitrogen ions covering an LET range of 20-470 keV μm^{-1} . Table 1 gives the coefficients of the dose-response curves for inactivation and mutation by these radiations (see Table 1 legend and ref. 5). The RBE_a is the ratio of the linear terms (α coefficients) of the respective dose-response curves. For hamster cells, however, the RBE varies with dose because the shapes of the dose-response curves change with LET (ref. 5). This is seen in Table 1 which gives values of RBE at low doses (RBE_a) and an example of values at higher doses (RBE_{HD}).

A plot of RBE against LET for the human cells is shown in Fig. 1 and for the hamster cells in Fig. 2. The variation of RBE with LET for inactivation and mutation induction in both cell types shows similar humped forms with maxima in the LET range 85-200 keV μm^{-1} . In this LET range the RBE for mutation induction was about twice that for inactivation. In hamster cells, this approximately twofold difference in RBE values was not influenced by the response level at which RBE was determined (Table 1).

The forms of RBE-LET relationships in Figs 1 and 2 can be explained if mutation or cellular inactivation require that one charged particle should leave either (1) several energy loss events in a single sensitive cellular component or target⁶⁻⁸ or (2) one energy loss event in each of two targets⁹⁻¹². On hypothesis (2) the two targets could be the two strands of a DNA duplex (diameter ~ 2 nm)¹⁰⁻¹².

If the dimensions of the target are small, then differences in the track structure of different ions of similar mean LET,

Fig. 1 RBE-LET relationships for freshly-isolated human diploid fibroblasts. Open symbols, mutation induction; closed symbols, inactivation. $\diamond$, $\blacklozenge$, 250-kVp X rays; $\circ$, $\bullet$, ^4He ions; $\triangle$, $\blacktriangle$, ^{10}B ions; $\square$, $\blacksquare$, ^{14}N ions. Data points are RBE_a values of Table 1. An arbitrary LET value has been assigned to X rays within the range given in ref. 1.



such as track width and LET spectrum, may influence the relative effectiveness of the radiation. Some of our data are consistent with this idea, as seen in the difference between the RBE for boron ions of $110 \text{ keV } \mu\text{m}^{-1}$ (track width $\sim 1,000 \text{ nm}$) and for helium ions of $90 \text{ keV } \mu\text{m}^{-1}$ (track width $\sim 40 \text{ nm}$) for the inactivation of hamster cells (Fig. 2). But, since these differences were not found for the human cells other mechanisms may be involved; for example, differences in the abilities of the two cell types to repair radiation damage^{13,14}.

There is some evidence from the work of others that the

different types of mutation induced by ionising radiation may have different RBE-LET responses. With high LET helium and carbon ions the maximum RBE for deletion mutations in *Neurospora* was greater than that for point mutations⁷. Similarly, in the *d-se* region of the mouse genome, more than twice as many multifunctional mutations were produced by fission neutrons than by X or γ rays¹⁵. These multifunctional mutations are believed to arise mainly as a result of deficiencies (deletions)¹⁵, suggesting a higher neutron RBE for deficiencies than for mutations at single loci.

Table 1 Mutation and inactivation of human and hamster cells by ionising radiations

a Inactivation		Human cells		Hamster cells			
Radiation type and LET		$\alpha \times 10^3$ (rad^{-1})	RBE _a	$\alpha \times 10^3$ (rad^{-1})	$\beta \times 10^6$ (rad^{-2})	RBE _a	RBE _{HD}
		Best fit (s.e.)		Best fit (s.e.)	Best fit (s.e.)		
X rays/ γ rays		7.92 (0.16)	1.0	1.43 (0.15)	2.6 (0.2)	1.0	1.0
Helium ions (LET in $\text{keV } \mu\text{m}^{-1}$)							
20		10.9 (0.2)	1.4	4.9 (0.3)	1.8 (1.2)	3.4	1.7
28		12.5 (0.5)	1.6	5.5 (1.1)	4.2 (3.1)	3.8	2.1
50		19.8 (0.3)	2.5	9.3 (1.0)	2.5 (5.4)	6.5	3.0
70		25.7 (0.5)	3.2	9.6 (1.4)	9.7 (7.9)	6.7	3.6
90		31.6 (0.7)	4.0	12.9 (0.8)	7.0 (5.1)	9.0	4.3
Boron ions (LET in $\text{keV } \mu\text{m}^{-1}$)							
110		29.6 (1.0)	3.7	7.4 (0.9)	14 (5)	5.2	3.2
160		27.9 (1.4)	3.5				
200		20.0 (0.3)	2.5	11.2 (2.1)	19 (20)	7.9	4.4
Nitrogen ions (LET in $\text{keV } \mu\text{m}^{-1}$)							
470		13.8 0.3	1.7	8.8 (0.4)	-1.9 (0.9)	6.2	2.5
b Mutation		Human cells		Hamster cells			
Radiation type and LET		$\alpha \times 10^7$ (rad^{-1})	RBE _a	$\alpha \times 10^7$ (rad^{-1})	$\beta \times 10^{10}$ (rad^{-2})	RBE _a	RBE _{HD}
		Best fit (s.e.)		Best fit (s.e.)	Best fit (s.e.)		
X rays/ γ rays		3.10 (0.08)	1.0	0.35 (0.05)	0.86 (0.15)	1.0	1.0
Helium ions (LET in $\text{keV } \mu\text{m}^{-1}$)							
20		3.94 (0.08)	1.3	1.0 (0.7)	3.6 (4.7)	2.9	2.3
28		4.85 (0.39)	1.6				
50		8.99 (0.33)	2.9	4.9 (0.6)	8.0 (5.2)	14	6.2
70		13.5 (0.6)	4.3				
90		22.0 (1.7)	7.1	6.0 (2.2)	41 (31)	17	9.5
Boron ions (LET in $\text{keV } \mu\text{m}^{-1}$)							
110		20.1 (1.4)	6.5	6.2 (2.7)	30 (27)	18	8.9
160		17.8 (1.2)	5.7				
200		20.7 (0.9)	6.7	6.2 (4.3)	33 (59)	18	9.1
Nitrogen ions (LET in $\text{keV } \mu\text{m}^{-1}$)							
470		8.5 0.3	2.8	5.4 (0.5)	-2.4 (2.0)	15	5.3

Cultures of freshly-isolated human fibroblasts (HF19) and an established line of Chinese hamster cells (V79-4) were maintained in Eagles minimal essential medium plus 10% foetal calf serum (Gibco-Biocult) using methods previously described^{20,21}. In most experiments cells were spread on to dishes with thin plastic bases (ICI Melinex), incubated overnight, and then irradiated without medium present. Techniques used for the various radiations are described in ref 5. Track intersection irradiations with ^4He , ^{10}B and ^{14}N ions were carried out at the variable energy cyclotron and the van der Graaf tandem accelerator of the AERE, Harwell. Methods of irradiation and dosimetry were similar to those of Neary²² except that the dishes were held in a wheel which rotated continuously during the irradiation to bring each dish in line with the beam a number of times. The average absorbed dose rate during each irradiation was $40\text{--}120 \text{ rad min}^{-1}$ which correspond to instantaneous absorbed dose rates of $2\text{--}9 \text{ krad min}^{-1}$ during the time that each dish was in the beam. The LET values are based on stopping power data for water assuming a density of 1 g cm^{-3} .

The survival of irradiated cells was usually estimated by their ability to grow and form colonies on the Melinex dishes after incubation periods of 7 d (hamster cells) or 12 d (human cells). Some survival estimations and all mutation estimations were made with cells respread after irradiation on to polystyrene dishes or bulk culture vessels²³ with normal growth medium. Mutants deficient in HGPRT activity were selected by resistance to the cytotoxic purine analogue 6-thioguanine⁴. To detect the maximum number of these mutants amongst irradiated cells, the cells were allowed different periods of post-irradiation growth in normal medium and then were respread into medium containing 6-thioguanine. Factors affecting the detection of radiation-induced mutants of this type have been described in detail elsewhere^{13,14,21,24}. For human cells the logarithm of surviving fraction and the induced mutation frequency per viable cell were both linearly related to dose, while for hamster cells these quantities were most simply described by a quadratic equation. Consequently the data points, weighted by the inverse of their variances, were fitted by the method of least squares to the following equations:

$$\begin{aligned} \text{hamster cell surviving fraction, } S/S_0 &= \exp -(\alpha D + \beta D^2) \\ \text{human cell surviving fraction, } S/S_0 &= \exp -(\alpha D) \\ \text{hamster cell induced mutation frequency, } M &= \alpha D + \beta D^2 \\ \text{human cell induced mutation frequency, } M &= \alpha D \end{aligned}$$

where D is the dose. The best fits for the coefficients α and β are listed above; the errors shown are simple standard errors (s.e.) of each coefficient in turn with the other at its best fit value. RBE values are derived either as the ratio of α for any given radiation to α for X or γ rays (RBE_a) or, for the hamster cells only, also as the ratio of doses required to produce 10% survival or an induced mutation frequency of 6.8×10^{-5} (this frequency is found after a dose of γ rays yielding 10% survival) (RBE_{HD}). The results given for helium ions of 20 and 28 $\text{keV } \mu\text{m}^{-1}$ and for nitrogen ions of 470 $\text{keV } \mu\text{m}^{-1}$ are from single experiments; two or more experiments were carried out for the remaining LETs.

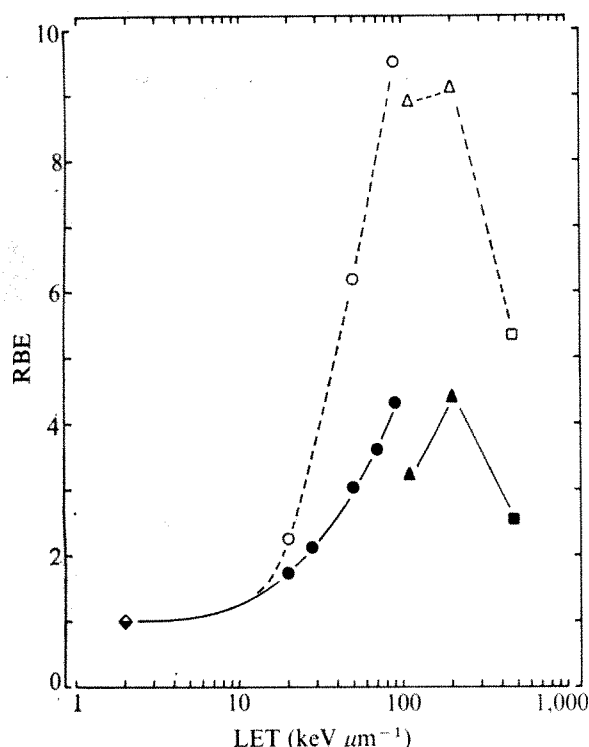


Fig. 2 RBE-LET relationships for established V79 Chinese hamster cells. Symbols as for Fig. 1 except $\diamond$, $\blacklozenge$, ^{60}Co γ rays. Data points are RBE_{HD} values of Table 1. An arbitrary LET value, comparable to that given for X rays in Fig. 1, has been assigned to γ rays within the range given in ref. 1.

No current genetic techniques for somatic cells can distinguish small deletions from point mutations. In man, however, the HGPRT gene is known to be located on the long arm of the X chromosome¹⁶ and gross genetic changes associated with loss of HGPRT may be detectable in mutants of cultured cells by chromosome banding techniques. Our preliminary results with ASG banding¹⁷ show that visible aberrations in the long arm of the X chromosome occur in about 40% of HGPRT-deficient mutants induced by nitrogen ions of high LET. In contrast, X-chromosome aberrations have not yet been detected in spontaneous and X-ray induced thioguanine-resistant mutants.

These preliminary observations suggest that an additional mutagenic mechanism becomes important with high LET irradiations. We previously found a simple relationship between inactivation and mutation induction by low LET radiation in cultured mammalian cells^{4,18}, and an additional mutagenic mechanism operating at higher LETs could explain the higher maximum RBE for mutation induction relative to inactivation (Figs 1 and 2). It is not yet possible to distinguish between two alternative molecular processes which might be involved in this additional mechanism: (1) that a different type of mutagenic lesion is induced by high LET radiation or (2) that lesions similar to those induced by low LET radiation are modified or interact after high LET radiation to give an increased probability of the lesions becoming mutagenic.

The implications for radiobiological theory of the data summarised here will be discussed more fully in subsequent publications. It is clear, however, that these data provide useful empirical confirmation of current radiological protection standards. The recommendations of the International Commission on Radiological Protection on maximum permissible doses for radiation effects in man include 'quality factors' (Qs) which are intended to allow for the effects of LET. The maximum Q is given as 20 (ref. 19), in agreement with the RBE for mutation induction by chronic irradiation in the mouse, and this is sup-

ported by the RBE_s obtained here for mutation of cultured hamster cells by low doses. The cultured human cells showed a maximum RBE for mutation induction somewhat less than this maximum Q. We should stress that the RBE values found for mutation to HGPRT-deficiency in cultured cells are probably averages of the RBEs for more than one type of genetic change, as discussed above. Consequently other mutations, perhaps comprising different combinations of genetic changes, might have RBEs different from those found here.

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Note added in proof: We now have evidence that X-chromosome aberrations are associated with thioguanine resistance in a proportion of mutants induced by both low and high LET radiations, but that different types of aberration predominate at different LETs.

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Group and mass effects in diprionid sawflies

THE larvae of gregarious insect species, when reared as single individuals, have a significantly higher death rate than do solitary forms¹. This has been demonstrated in diprionid sawflies, where all main genera have solitary and very gregarious species². It has been argued that this high mortality is due to starvation, because only one in three of the newly hatched larvae of the gregarious *Neodiprion pratti* could feed successfully on the tough pine needles, whereas larvae in a colony could join and enlarge feeding sites, thus benefiting from an elementary 'division of labour'³. Our experiments, however, have not confirmed such a mechanistic explanation.

Improved rearing techniques kept larval mortality at ~10% for most species, including the solitary *Diprion frutetorum* and the scarcely gregarious *N. abietis*. A consistently high mortality was encountered only in individually reared first instars of *N. lecontei*, which is undoubtedly the most gregarious species we have studied. The most unexpected results were the very low mortality of isolated first instars of the two gregarious species *N. rugifrons* and *N. pratti* (Fig. 1). Although most deaths in these experiments occurred in the first instar, they were not, as has been claimed, caused by starvation. More than 70% of the larvae had fed before they succumbed. Paradoxically, it

was the doomed isolated first instars of gregarious forms that produced many more feeding incisions than did the larvae that were reared in groups (Fig. 2). Psychological factors entered into the situation to change the behaviour of the larvae. These were indicated by an increased restlessness of the larvae which wandered for long periods between each feeding bout and sometimes left the foliage altogether. This disjointed behaviour could be stabilised by the addition of more larvae to the experimental situation, until a species-specific group size was attained when feeding became normal and mortality was low. This threshold was reached with five larvae in *N. lecontei* on red pine, but with a smaller group size on jack pine, the optimal host available to this group (Fig. 1).

Colonial feeding in social animals is often associated with better weight gain and thus shorter rates of development. No such effect was noticed in *N. lecontei* when isolated and group-reared animals were compared. Singly reared *N. pratti* larvae took 30% more time to complete their feeding stages than their brother and sister reared in aggregations. The ecological implications of a mechanism that reduced the exposure time to enemies are obvious, although by no means the only benefits accruing from a gregarious habit. The larvae discourage their attackers by the discharge of a viscous, repellent liquid and the synchronous beating of head and thorax. These activities not only result in reduced losses but also in a disproportionately high survival of individuals in the centre of the colony, at the expense of those located at the periphery⁴.

The intensity of aggregative behaviour in diprionid larvae is eclipsed by other sawflies, many of them confined to the Southern Hemisphere. Females of the Brazilian *Themos olfersii* (Argidae)⁵ and the Australian *Pseudoperge guerini*⁶ guard not only their eggs but also their young larvae for several weeks. Cooperation in the *Pseudoperge* larvae is especially important just after hatching when they have to fight their way through the tough epidermis of the *Eucalyptus* leaves in which the egg pods are placed. Similarly, mature larvae have to penetrate the hard crust of the soil to spin their massed cocoons underground. Larger groups clearly succeed much better at both tasks. Cohesiveness and conspecific attraction of *Pseudoperge*

Fig. 1 Larval mortality of *Neodiprion* spp. in six experimental situations. A group effect was observed in *N. lecontei*, where an increase in the feeding aggregations resulted in a linear decrease in mortality up to a species-specific group size (which was five larvae on red pine, the natural host, but only four on jack pine). *N. abietis* showed a mass effect, whereas mortality in *N. pratti* was unrelated to group size. ●, *Neodiprion lecontei* on red pine (*Pinus resinosa*); ○, *N. lecontei* on jack pine (*P. banksiana*); ▲, *N. pratti* on jack pine; ■, *N. abietis* on balsam fir (*Abies balsamea*).

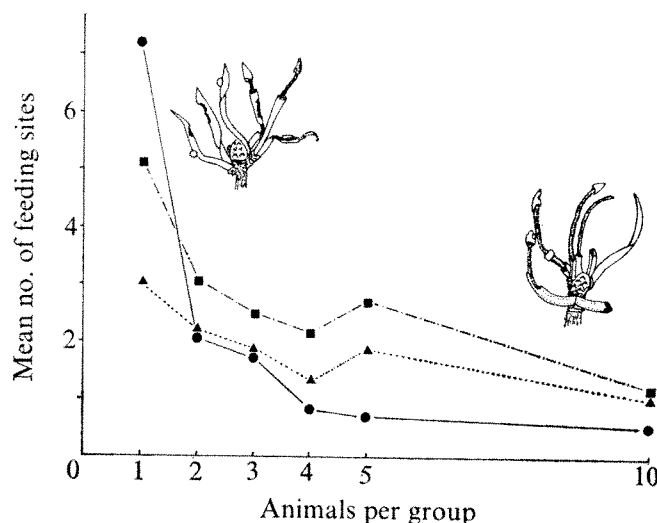
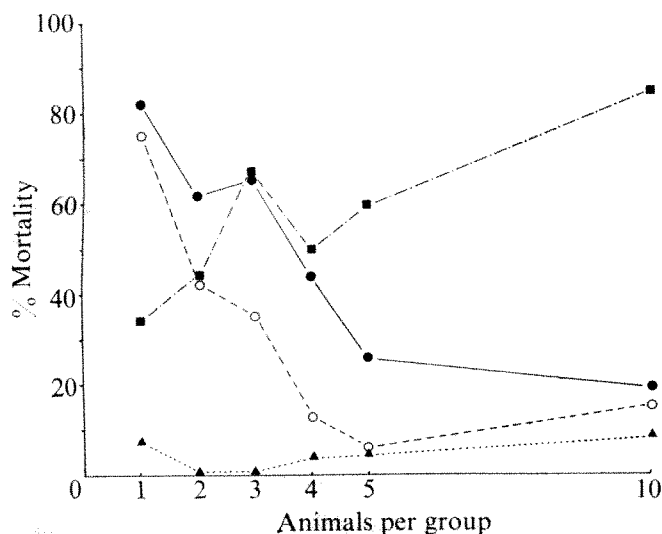


Fig. 2 Feeding patterns of *Neodiprion* larvae 1–6 d old. The mean numbers of feeding sites per larva were significantly different for each species ($P < 0.001$). Large feeding aggregations resulted in normal feeding patterns. In small groups the gregarious *N. lecontei* showed disjointed behaviour resulting in scattered feeding sites (compare the two drawings) accompanied by high mortality (Fig. 1). The difference in number of feeding sites is significant ($P < 0.001$) between groups of three and four larvae for *N. lecontei*, whereas for *N. abietis*, the scarcely gregarious species, such a difference exists only between one larva and a group of two. These differences correspond to species-specific group sizes. No such effects were observed for *N. pratti*, thereby explaining its low mortality. ●, *N. lecontei*; ▲, *N. pratti*; ■, *N. abietis*.

larvae is unrivalled by Northern Hemisphere sawflies. Fifty larvae of all ages spaced regularly on a paper grid 50 cm × 50 cm coalesced within 25 min to form an intertwining mass inseparable without the use of anaesthesia. Gregarious *Neodiprion* larvae by contrast, showed only feeble interest in one another when similarly tested. The presence of food caused the formation of large, stable groups but even dry, inedible foliage encouraged temporary groups of two or three larvae to be formed.

Although gregarious behaviour, or its absence, has led to obvious morphological and behavioural modifications, it seems that the greatest impact for most species concerns their ecology and population dynamics. The tenthredinid sawfly genus *Pikonema* illustrates this hypothesis well. Two related species feed on spruce, but whereas *P. alaskensis* is common and kills trees, *P. dimmockii* is never abundant or a threat to its host and the reason for this is found in the behavioural patterns of the larvae. The gregarious *P. alaskensis*, unlike the solitary *P. dimmockii*, does not get alarmed by frequent contacts with other larvae but resumes feeding soon after contact. *P. dimmockii* larvae wander for long periods after each encounter and settle down only with difficulty⁷. This suggests that two opposite types of density-dependent adaptations have evolved in larval sawflies. The reciprocal sensory stimulation in the larvae of gregarious species causes a group effect⁸ that enhances the optimal development. A similar stimulation creates a mass effect⁹ with often lethal consequences in solitary species. Similar subtle pressures seem to have operated in the rearing experiments with *N. abietis* (and *D. frutetorum*), where a clear relationship was established between increasing population density and higher larval mortality (Fig. 1). The primitive sociality of some gregarious species is of special interest since interactions between group mates of a feeding aggregation may eventually explain the emergence of cooperation in the complex societies of wasps, bees and ants.

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Oestradiol and its biosynthesis in *Phaseolus vulgaris* L.

UNTIL recently the presence of steroidal oestrogens in plants has been a matter for debate¹, and any physiological significance associated with them has been generally disregarded^{2,3}. Although steroidal oestrogens have been found in extracts of a few species⁴, the identification of oestrone in *Phaseolus* was based on equivocal procedures⁵. Moreover, as with any compound present in low concentration, it is possible that at least some of the oestrogens identified in plant extracts are attributable to external contamination. This risk can be eliminated by demonstrating the incorporation of radioactive label from a known precursor into the compounds of interest. We report here the incorporation of radioactive isotopes from (3RS)-2-¹⁴C-mevalonic acid DBED (MVA), 4-¹⁴C-oestrone and potassium 6,7-³H-oestrone sulphate into oestradiol by *Phaseolus vulgaris* L., cultivar Canadian Wonder. This represents the first case known to us of successful labelling of an identified steroidal oestrogen from ¹⁴C-MVA in a higher plant. It seems therefore that this class of mammalian sex hormone is produced in plants as well as animals.

Eight-day-old seedlings of *P. vulgaris* were sectioned under water at the base of the hypocotyl, stood in aqueous solutions of the various radioactive substances and allowed to transpire for 5 d (ref. 6). They were then planted in potting compost and grown for 2–5 weeks to facilitate normal growth and development. Harvested plants were solvent extracted (chloroform:methanol:2:1) under reflux in a Soxhlet apparatus for 8 h. The extract was reduced to dryness *in vacuo* and taken up in ether. The phenolic fraction was isolated by partitioning with 1N NaOH, followed by acidification of the aqueous phase and repartitioning into ether. Thin-layer chromatography (TLC) and column chromatography on Sephadex LH-20 were used to isolate those fractions expected to contain oestrone (E₁) and oestradiol (E₂).

The chromatographic mobilities of metabolites were compared with authentic compounds using TLC on three solvent systems (chloroform:methanol:95:5; benzene:propan-2-ol:95:5; petroleum ether:ethyl acetate:75:25), column chromatography on LH-20 of the underivatized extracts and radio-gas-liquid chromatography (radio GLC) on OV-101 of the trimethylsilyl ether derivatives. Samples believed to contain oestradiol were diluted with non-radioactive carrier oestradiol and recrystallised to constant specific activity.

Using these techniques in combination, ¹⁴C was observed to be incorporated into oestradiol from MVA and from 4-¹⁴C-oestrone. Similarly, oestradiol could be labelled from the

³H-oestrone sulphate. Attempts to label oestrone have not been conclusive; the rate of incorporation from radio-active MVA into the putative oestrone peak was much less than that observed for oestradiol.

One previous report describes the incorporation of ¹⁴C-MVA into a phenolic fraction by the higher plant *Haplopappus heterophyllus*, but the radioactive phenolic material did not correspond to any common steroidal oestrogen⁷. There have been many studies of the biotransformation of the mammalian steroid hormones progesterone and testosterone^{4,8}, but our findings seem to represent the first report of a metabolic transformation of an oestrogen in plants—reduction of the 17-keto steroid E₁ to the 17β-hydroxyl compound E₂.

Evidence of unlabelled oestradiol in extracts of seeds, vegetative and flowering plants was obtained using a modified radioimmunoassay (RIA) technique⁹ and combined gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis¹⁰ involved continuous monitoring at the collector of the ion abundance at m/e 416 (molecular ion of the bis-trimethylsilyl derivative of oestradiol) as generated in the ion source from the GLC effluent of an SE-30 column operated at 250 °C. This process, commonly referred to as single-ion monitoring, enabled us to use the GC-MS system as a semi-specific detector for oestradiol.

This identification contrasts with the results of Kopcewicz⁵ who presented TLC evidence for oestrone in *P. vulgaris* but could not detect oestradiol in the extracts. Indeed, this is only the second report of oestradiol detected as a plant steroid, Awad¹¹ having isolated both oestrone and oestradiol from seeds of *Prunus*.

The verification of the endogenous biosynthesis of a steroid oestrogen in *P. vulgaris* opens the way to further studies of the suggested correlation between the quantitative levels of these compounds in this species and the onset of flowering⁵. Preliminary data obtained by RIA suggest that oestradiol is present at 2–10 μg kg⁻¹ fresh weight in seeds and leaves. It should also be possible to examine the overall pathway of biosynthesis of steroids in plants in order to investigate the presumed homology between plants and animals⁴. In addition, the potential for hormonal heterophyly¹² with respect to oestrogens in plants requires attention.

We thank Dr B. Cook for assistance with RIA and Professor K. Overton and J. Dalziel for radio-GLC facilities. The GC-MS instrumentation (AEI MS30) was provided by grants from the SRC, and I.J.Y. was financed by a post-graduate award from the SRC.

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Direct evidence of the capacity of the XY germ cell in the mouse to become an oocyte

INDIRECT evidence of the capacity of a Y-containing germ cell to become functional oocyte in ovarian tissue of a chimaeric XX/XY female mouse has been published¹. This animal was one of two fertile XX/XY females obtained by aggregation of early morulae of the inbred strains AKR (albino) and CBA-T6 (agouti). Mated to a BALB/c (albino) male she had given birth to two litters that included one albino son, the remaining six young being agouti. Afterwards she had been retained for observation in connection with another investigation. In consequence she was more than 12 months old when her chromosomes were examined and she was found to contain both XX (CBA) and XY (AKR) cells. Only then was it realised that this implied that the egg that gave the albino son had come from a Y-bearing oocyte. Although ancillary evidence effectively excluded the possibility of erroneous recording or mixture of animals as explanations, there may have been some reluctance to accept the conclusion that a Y-bearing germ cell had become a functional oocyte. It is therefore satisfying to report here the direct observation of an XY oocyte at diakinesis in a preparation from another fertile, chimaeric XX/XY female mouse.

The animal was one of a series of chimaeras obtained by aggregation of embryos from matings chosen with another purpose in view². When breeding tests and other observations had been completed cultures were set up from tail blood of some of the animals and two females that had bred successfully were found to be XX/XY chimaeras. The one (M 9263) had an XY 'pigmented' component with a standard karyotype and an XX 'albino' component marked by a single large metacentric chromosome (Rb1 Ald); the other (M 9290) had an XX 'pigmented' component and an XY 'albino' component homozygous for Rb1 Ald. Both underwent a more detailed chromosome study that included various somatic tissues and also meiosis in oocytes, using preparations made by a modification of Tarkowski's³ method following brief *in vitro* culture⁴. One oocyte from M 9263 at diakinesis (Fig. 1) contained 19 normal bivalents, plus two univalents corresponding closely in relative size to the univalent X and Y chromosomes seen frequently in spermatocytes. Any doubt that these two univalents may have represented a deficient chromosome (not necessarily X) and its normal homologue is removed by the presence of 19 normal bivalents, and the absence of the Rb1 Ald trivalent seen in the remaining oocytes (Fig. 2).

The discovery of this XY oocyte in diakinesis, we hope,

Fig. 1 Oocyte at diakinesis from XY component of M 9263. 19 bivalents plus X and Y univalents (arrowed). Bar represents 10 nm.

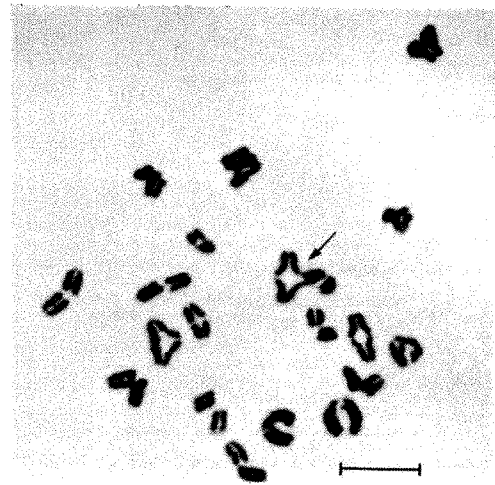
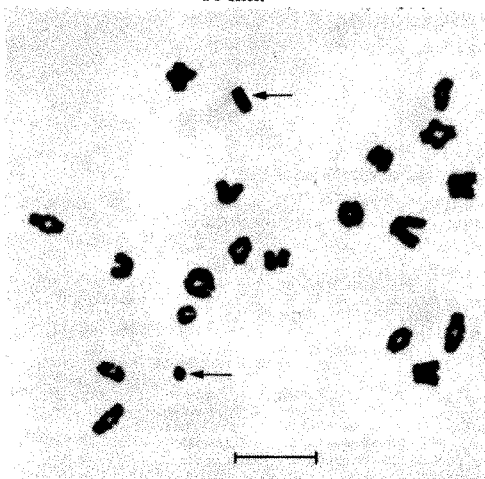


Fig. 2 Oocyte at diakinesis from XX component of M 9263. 18 bivalents plus Rb1 Ald trivalent (arrowed). Bar represents 10 nm.

will remove any scepticism that may remain concerning origin of the exceptional albino son of the earlier report from a Y-bearing oocyte. The cytogenetic evidence showed that the son had received his Y chromosome from his mother and also, surprisingly, that he had the rare 41, XXY ('Klinefelter') karyotype¹. The possibility could therefore not be excluded that a chance non-disjunctional event during oogonal mitosis had given an XXY oocyte that had contributed an X and a Y chromosome to the zygote by secondary non-disjunction.

The alternative was to assume primary non-disjunction in an XY oocyte. Should the first explanation be correct, survival and maturation of the XXY oocyte may have been favoured, even made possible (in view of the presence of the Y chromosome), by the presence of the two X chromosomes, and the event could then have been unique and of trivial significance. The new observation, however, shows that an XY oocyte can at least develop to the point where its germinal vesicle is capable of resuming meiosis during the brief period (4 h) of culture *in vitro*. This normal response to culture and the normal appearance of the chromosomes, moreover, strongly suggest that the oocyte was developing naturally and that it would have been capable of completing normal maturation, including release from its follicle, transport to the oviduct and fertilisation.

The presence of univalent X and Y in the exceptional oocyte (see Fig. 1) gives a hint, and only a hint, that pairing of X and Y in oocytes may be less assured than in spermatocytes, thereby increasing the likelihood of non-disjunction and rendering more plausible the assumption of primary non-disjunction in an XY oocyte.

Relevant data from the four known fertile XX/XY females are summarised in Table 1. The 22 scorable oocyte preparations were obtained from 25 oocytes set up in culture. Breeding records and direct cytological examination together indicate actual or potential functional capacity of 173 XX oocytes and two XY oocytes. This gross disproportion may at first suggest that XY germ cells, though capable of normal function, are nevertheless at a strong disadvantage compared with XX germ cells. Further consideration, however, indicates that such a conclusion would be premature.

The literature provides records of 36 XX/XY chimaeric mice constructed by aggregation of morulae and representing balanced combinations⁵ of component strains: 21 of them were fertile males, six sterile males, three intersexes, four were sterile females, and only two were fertile females⁶⁻¹⁰. Most XX/XY chimaeras evidently develop as males and it is reasonable to suppose that the XX/XY

Table 1 Observations on fertile XX/YY female chimaeras

Mouse number	Age when killed (months)	Sex chromosomes and estimated percentage composition of soma		Offspring		Sex chromosomes of oocytes	
		Pigmented component	Albino component	Pigmented	Albino	XX	XY
9263	13	XY 2	XX 98	0	64	12	1
9290	13	XX 82	XY 18	66	0	9	0
15	15	XX 65	XY 35	6	1	} Not studied	
16	17	XX 75	XY 25	16	0		

The chimaeras were obtained by aggregation of morulae from pigmented and albino stocks. The percentage composition of the soma was estimated as the mean of estimates of proportions of mitotic cells of the two types in direct chromosome preparation from the lymphomyeloid complex, corneas and gut epithelium (mouse 9263); or from lymphomyeloid complex and corneas only (mouse 9290); or the percentage of agouti in the coat (mice 15 and 16)¹. The offspring were derived from matings to albino males. The sex chromosomes of the oocytes were identified in direct chromosome preparations.

females are those that, by chance, contained relatively few XY cells as embryos. It is therefore not surprising to find that the mean proportion of the XY component in the soma, derived from the estimates for the four individual XX/XY females (Table 1), is only 20%. If the proportion of XY cells is assumed to be the same in germ line and soma, the probabilities of drawing a single XY oocyte (or none) in random samples of 77 (chimaera 9263), and seven (chimaera 15), are given by the sums of the first two terms of the binomials $(0.98+0.02)^{77}$ and $(0.65+0.35)^7$, and are 0.54 and 0.23 respectively. Furthermore, breeding tests show that even in XX/XX and XY/XY chimaeras the germ cells are frequently derived from only one of the two components¹¹. In the series from which M 9263 and M 9290 were taken, only eight of the 15 female chimaeras had mixed progenies. Of the remaining seven, one died before tests were completed and four proved on chromosomal examination to be XX/XX, only the two animals described here being XX/XY. The lack of evidence of functional XY ova in two of the four fertile XX/XY females would not therefore be remarkable even if XX and XY oocytes had equal potential for maturation and function.

The rarity of functional XY oocytes in fertile XX/XY female chimaeras cannot therefore yet be taken to indicate that the XY germ cell is at a disadvantage of some kind when it finds itself in a potentially competitive position with XX germ cells either during early proliferation and migration in the developing embryo or later in the ovarian environment. A disadvantage would nevertheless not be surprising in view of the evidence that both X chromosomes are normally active in XX oocytes¹²⁻¹³, and that early embryos derived from XO mothers develop more slowly than those derived from XX mothers¹⁴.

The decisive demonstration that XY germ cells can become functional oocytes means that the developmental fate of the primordial XY germ cell is not irrevocably determined before its migration to the gonads is complete. Evidently the sex of a germ cell is not an autonomous property but is determined by the nature of the gonad in which it finds itself. Our demonstration provides further evidence to counter recent criticism¹⁵ of our original report.

We thank P. H. Glenister for construction of chimaeras.

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The XY chromosome pair in mouse and human spermatocytes, visualised by silver staining

THE nature of the relationship between the X and Y chromosome in human spermatocytes has not been clearly explained by the extensive cytological studies of the last 50 yr (see refs 1-3 for reviews). The sex chromosomes of man and other mammals form a condensed mass (the so-called 'sex vesicle') during zygotene-pachytene in spermatocyte nuclei. Because of its density, the internal structure of the sex vesicle could not previously be studied by light microscopy. We describe here a technique which clearly stains the sex chromosomes within the condensed sex vesicle.

Testicular biopsies of C57BL/6 and BALB/c mice, and of human males were processed by the technique of Evans *et al.*⁴. The material was placed in 1% sodium citrate, teased apart with dissecting scissors to free the tubules, and left for 1 h. After centrifugation, the supernatant was gently pipetted off and the pellet fixed with a fresh mixture of 3:1 methanol:acetic acid for 30 min. The fixative was changed twice. Chromosome preparations were made by the air drying technique.

The silver staining procedure is simplified from a technique of Howell *et al.*⁵, developed to demonstrate nucleolus organiser regions on somatic chromosomes. It consists of two basic steps: (1) Air-dried preparations are treated with three or four drops of 33% aqueous AgNO₃, pH 5.5, under a coverglass for 6-10 min beneath a photoflood light at a temperature of 50-70 °C. After cooling, the AgNO₃ is washed off with deionised water and the preparation is again air dried. (2) Working at room temperature, two drops of ammoniacal silver (8 g AgNO₃ dissolved in 10 ml H₂O, then added to 15 ml concentrated NH₄OH) are placed on the chromosome preparation and immediately followed by two drops of 3% acetate-neutralised formalin. A cover glass is added. The pH of the combined fluids should be 12-13. As soon as the preparation turns a deep yellow (10-60 s) the silver solution is quickly washed off, and the slide made permanent.

After this treatment, the sex vesicle can be seen as a prominent structure, situated at the periphery of the nucleus, and frequently slightly dissociated from it (presumably by the hypotonic treatment).

In the mouse, the two sex chromosomes are seen as thin, positively stained threads, each almost symmetrically curled back on itself, the longer (presumptive X) being readily

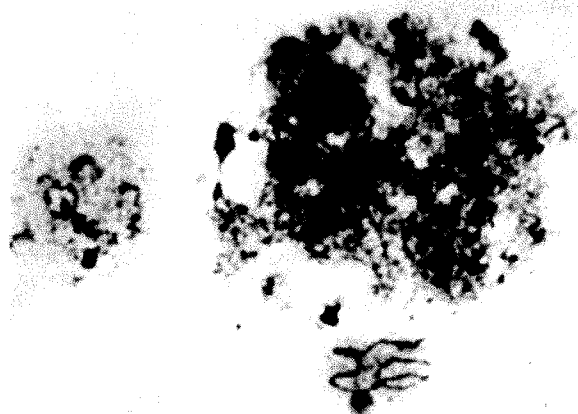


Fig. 1 Pachytene nucleus from a male mouse. X and Y chromosomes are clearly seen within the sex vesicle; also the nucleolus-like body associated with X.

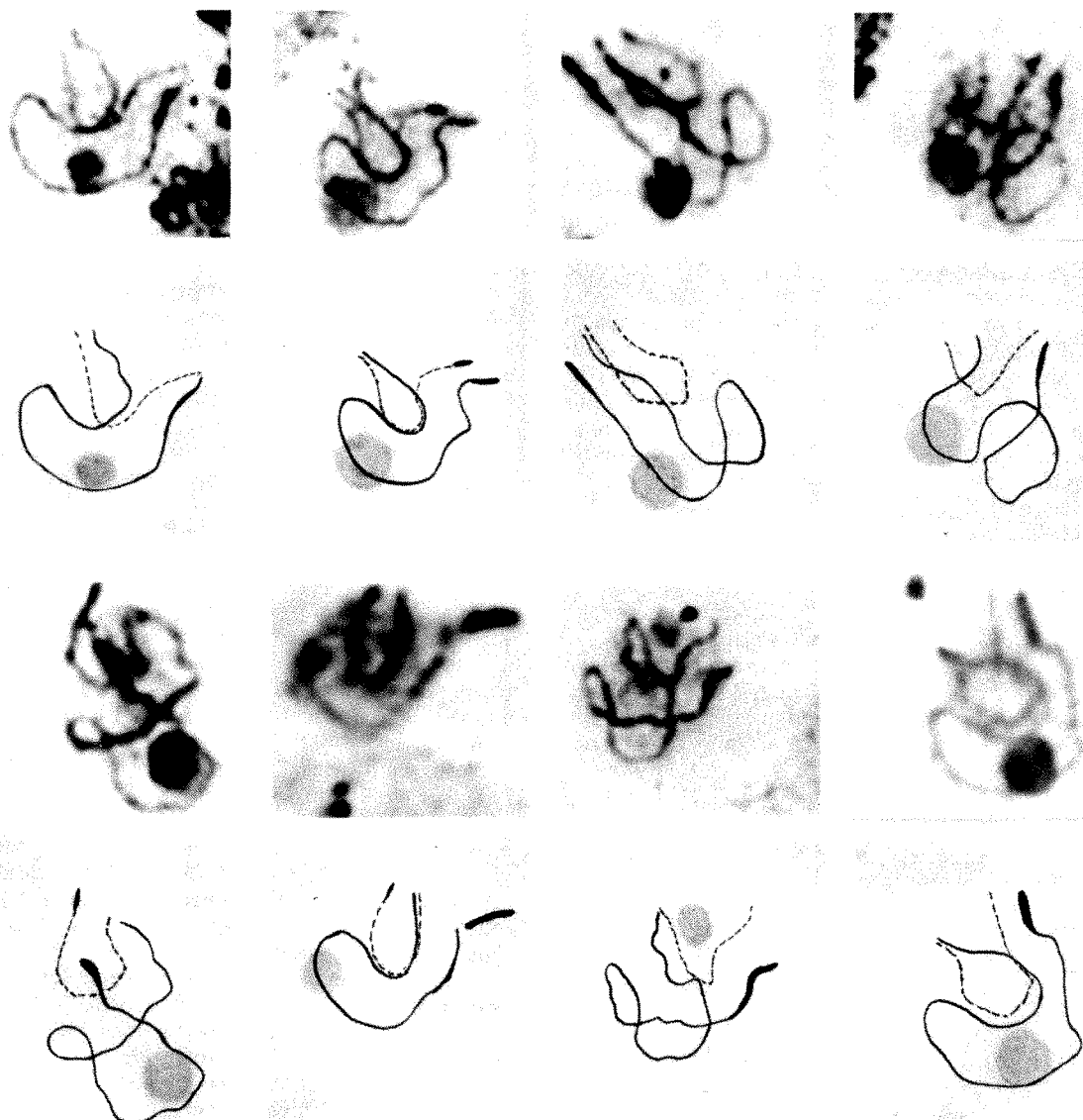
distinguished from the shorter (presumptive Y). Both ends of both chromosomes usually seem to be fixed to the periphery of the sex vesicle not very far from one another. One end of the X, and one end of the Y, are particularly

close to one another, and here the chromosomes seem to run in parallel for a short distance in what may be a parasyntaptic association. In addition to this association, the Y in many cells crosses the X again at approximately the same place along its length (see Figs 1 and 2).

At a point about one third along its length the X chromosome is quite regularly associated with a round body which looks like a nucleolus. At pachytene this sphere stains lighter than the surrounding material, although with acridine orange it looks very pale.

In man, the figure has features in common with that of the mouse, although in the early stages of meiotic prophase the sex chromosomes are curiously intertwined and although no nucleolus is evident in the vesicle. The sex bivalent is folded so that the X chromosome forms a rod with the presumptive centromere near the nuclear membrane, and the distal tip is associated with the Y chromosome. Synapsis between the long arms of both chromosomes would then be particularly easy (Fig. 3). In later stages, the bivalent looks much as it does at diakinesis, but more elongated. Generally, a third of the long arm of the X chromosome runs alongside the long arm of the Y chromosome. The X centromere and the distal part of the long arm of the Y are clearly seen, while the other bivalents are still almost indistinguishable.

Fig. 2 Sex bivalents from spermatocytes of the mouse at pachytene and interpretative diagrams. In the diagrams the X chromosomes are shown as continuous, the Y chromosomes as discontinuous.



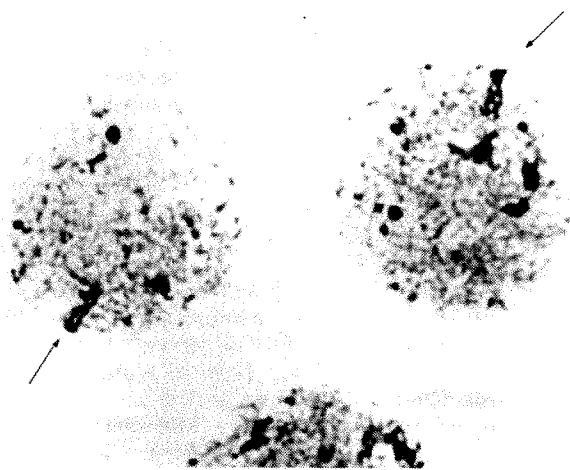


Fig. 3 Sex bivalents (arrowed) in spermatocytes from a normal human male at pachytene.

The sex vesicle is usually densely stained, but in favourable circumstances the curled up sex bivalent has been seen within it⁶. Our observations confirm the side-by-side configuration of segments of the sex chromosomes suggested by previous work. The pictures obtained by this relatively simple light microscope technique are very similar to those previously obtained by three dimensional reconstructions of electron microscope sections³.

The question about the existence of chiasmata between the human X and Y chromosomes has not yet been resolved². After diplotene, the sex pair is seen to be joined in an end-to-end relationship, which may be a terminalised chiasma. A common end region expressed as a short synaptonemal complex is present in the mouse and the human².

Detailed studies of the sex vesicle in pachytene by the method we have described could aid the analysis of structural rearrangements of the sex chromosomes in the mouse and other mammals and might complement clinical diagnosis in males with sex chromosome aberrations or infertility (Fig. 4).

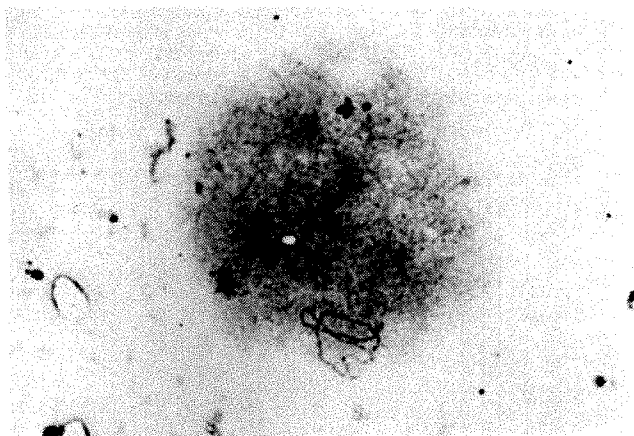


Fig. 4 Sex bivalent in a spermatocyte at pachytene from an azoospermic human male.

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Simpler technique for serological detection of H-Y antigen on mouse lymphocytes

THE serological detection of the H-Y (male) antigen in the mouse has been demonstrated¹ using the cytotoxicity test against mouse spermatozoa, and that work has been confirmed² by the demonstration of H-Y on male (not female) mouse epidermal cells. There are, however, technical difficulties associated with both assays and these are hard to overcome. We report here a simplified method for the serological detection of H-Y antigen on mouse lymphocytes using *Staphylococcus aureus* (SA) as a marker. We do this in the interests of other investigators who may wish to investigate the H-Y system by a relatively simple method.

SA contains protein A, a polypeptide of molecular weight 42,000 which binds to the Fc of IgG molecules from several species³. It is linked covalently to the peptidoglycan in the cell wall of most strains of SA⁴. Kearney *et al.*⁵ have described a method of detecting membrane-associated antigens on lymphoid cells by antibody coupled to protein A of SA. We have used this procedure to detect H-Y antigen expressed on male lymph node cells.

Mice were purchased from the Jackson Laboratory. Anti-H-Y serum was produced in C57BL/6 (B6) female mice by repeated inoculation with 25×10^6 B6 male spleen cells. Serum was obtained 10 d after the seventh inoculation. Anti-H-Y activity was determined by the cytotoxicity test, with mouse spermatozoa as target cells according to the method of Goldberg *et al.*¹. The Cowan I strain of SA containing 1.7% protein A was obtained from Dr R. C. Williams Jr. It was cultured in Todd-Hewitt broth (Difco) at 37 °C and collected after an 18-h incubation on a shaker. The cells were washed three times in phosphate-buffered saline (PBS; 0.02 M PO₄; 0.15 M NaCl, pH 7.2) and then incubated for 3 h at 37 °C in PBS containing 0.5% formaldehyde. The cells were then washed four times in PBS and placed in a boiling water bath (94 °C in Albuquerque, New Mexico) for 2.5 min at a 10% (v/v) cell concentration. This stock suspension was stored at 4 °C in the presence of 0.1% NaN₃. Before use, cells were washed and resuspended in PBS.

The axillary and inguinal lymph nodes were removed from B6 mice. After mincing into a single cell suspension in Eagle's minimal essential medium (MEM, Gibco), they were washed and resuspended to a concentration of 2×10^7 cells per ml. Anti-H-Y (50 µl) serum was added to 0.25 ml of lymph node cells from male mice and incubated for 30 min at 4 °C. To test for specificity of the H-Y antiserum, lymphocytes from B6 female mice were also treated with anti-H-Y serum. A further control consisting of male lymph node cells treated with normal mouse serum was also included. After washing twice with 5 ml (20 volumes) of MEM containing 10% foetal calf serum (FCS; previously heated to 56 °C for 30 min), the cell pellet was mixed with 0.05 ml of SA suspension and incubated for 15 min at 4 °C. To reduce the background number of unattached SA, the mixture was washed three times in 5 ml of MEM, containing 20% FCS, by centrifugation at 200g for 10 min at 4 °C. After the final wash, the pellet was resuspended in 0.25 ml of MEM. Smears were prepared on microscope slides, air dried, fixed in methanol and stained by the Giemsa method. The slides were observed by light microscopy at $\times 400$ and $\times 1,000$.

A lymphocyte was counted positive if five or more SA were attached. Male lymphocytes were never less than 90%

positive. The average background of positive female lymphocytes, with unabsorbed serum, was 10%, with an upper limit of 20%. With the technique described here, we have made eight tests, in each of which male and female lymphocytes were compared. The slides were coded and read by four individuals. All male cells typed H-Y⁺ and all female cells types H-Y⁻, and all readers agreed on all positive and negative readings.

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Tamoxifen is a potent 'pure' anti-oestrogen in chick oviduct

OESTROGEN responsive cells contain a specific proteinaceous macromolecule—one receptor—capable of recognising physiologically active oestrogens as well as a number of synthetic oestrogen agonists and antagonists^{1,2}. In the non-stimulated state, the majority of receptors are located in the cytoplasmic fraction of responsive cells^{3,4}, including the chick oviduct⁵. When oestrogen reaches the target cell, it binds to the cytoplasmic receptor causing its 'activation' and the translocation of the hormone-receptor complex to the nucleus. The interaction of receptor with chromatin is thought to facilitate gene transcription and the synthesis of proteins implicated in the changes characteristic of the oestrogenic response^{6,7}. Receptor is retained in the nucleus for varying periods of time depending on the concentration and nature of the oestrogen. In the rat uterus, the length of time that receptor is retained in the nucleus correlates well with a number of parameters of the hormonal response⁸. The initial translocation of receptor to the nucleus is followed by a period of cytoplasmic receptor replenishment^{3-5,9}. This process is very important as it has been implicated in the ability of cells to respond to subsequent oestrogenic stimulation^{10,11}. Anti-oestrogens prevent oestrogens from expressing their full effects on oestrogen target tissues. Recent studies have indicated that some non-steroidal anti-oestrogens (CI-628, nafoxidine and clomiphene) bind to cytoplasmic oestrogen receptor, induce the translocation of the antagonist-receptor complex to the nucleus, but fail to stimulate subsequent cytoplasmic receptor replenishment¹²⁻¹⁴. Oestrogen antagonism would be primarily due to inhibition of cytoplasmic receptor replenishment¹². Further, such a hypothesis could explain the weak oestrogenic activity of these compounds following a single injection when cytoplasmic receptor is translocated to the nucleus, and their subsequent potent anti-oestrogenic activity after serial injections when the low cytoplasmic receptor level is the limiting factor in receptor translocation¹⁵. We present here results which illustrate that this theory of anti-oestrogenic action does not hold for tamoxifen (ICI 46,474) in the chicken oviduct.

Oestrogen withdrawn (4-6 weeks) chickens^{5,16} were

injected with oestradiol benzoate 1 mg per kg body weight and/or tamoxifen 10 mg per kg. Magnum wet weight, DNA content, and cytoplasmic progesterone receptor concentration (increasing after oestrogen administration^{17,18}) were measured either 24 h after a single injection or 24 h after the last of three daily injections. All experimental procedures have been described previously^{5,18}. The simultaneous administration of oestradiol benzoate (1 mg per kg) and tamoxifen (10 mg per kg) resulted in complete abolition of the oestradiol benzoate induced responses. At the dose tested, tamoxifen alone demonstrated no oestrogenic activity (Fig. 1). Further experiments (manuscript in preparation) showed that even at a dose of 1 mg per kg, tamoxifen was capable of causing a significant ($\geq 50\%$) reduction in oestradiol benzoate induced magnum growth and cytoplasmic progesterone receptor concentration. Moreover, tamoxifen is a potent inhibitor of oestradiol induced ovalbumin synthesis, while having no detectable agonist effect of its own (Binart and Lebeau, unpublished).

Although tamoxifen did not induce any measurable oestrogenic response, it led to appreciable changes in the subcellular distribution of oestrogen receptors with the majority of receptor sites being located in the nucleus 24 h after a single injection (Fig. 2). No change in the total cellular oestrogen receptor level was observed at 24 h, in contrast to the increase after oestradiol benzoate and in agreement with the lack of oestrogenic properties of the anti-oestrogen. When the time-dependence of oestrogen receptor distribution was monitored over a 24 h period following injection of tamoxifen 10 mg per kg, it was apparent that approximately 70% of the cytoplasmic receptor was rapidly translocated to the nucleus during the first hour, and that this pattern of distribution was maintained for the entire period studied (Fig. 2a). In this respect, tamoxifen in the chick seemed to behave in a similar manner to the non-steroidal anti-oestrogens in the rat¹²⁻¹⁴, that is, long-term retention of the nuclear receptor and no (or delayed)

Fig. 1 The effect of oestradiol benzoate (E₂B) 1 mg kg⁻¹ and/or tamoxifen (Tam) 10 mg per kg on magnum wet weight (Wt), DNA content (DNA) and cytoplasmic progesterone receptor concentration (PR) 24 h after the final injection. Groups of oestrogen withdrawn chickens (4-6 animals per group) received either 3 injections at 24 h intervals (open bars) or a single injection (full bars). The data represent the mean $\pm$ SD of three groups of chickens and are presented as a percentage of values in non-injected control animals.

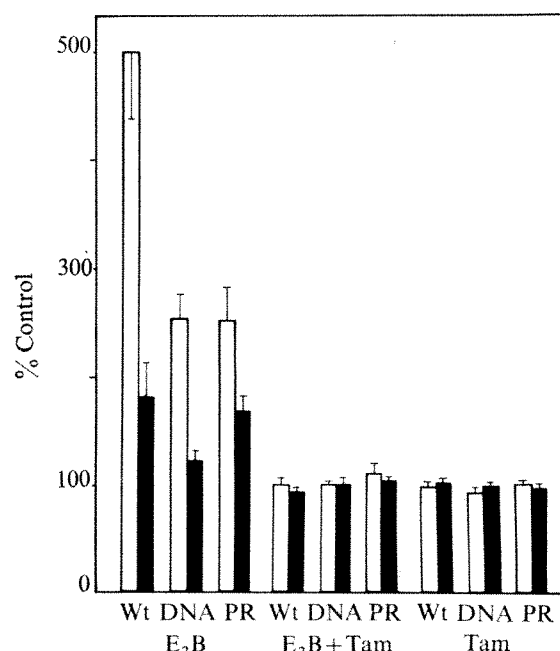
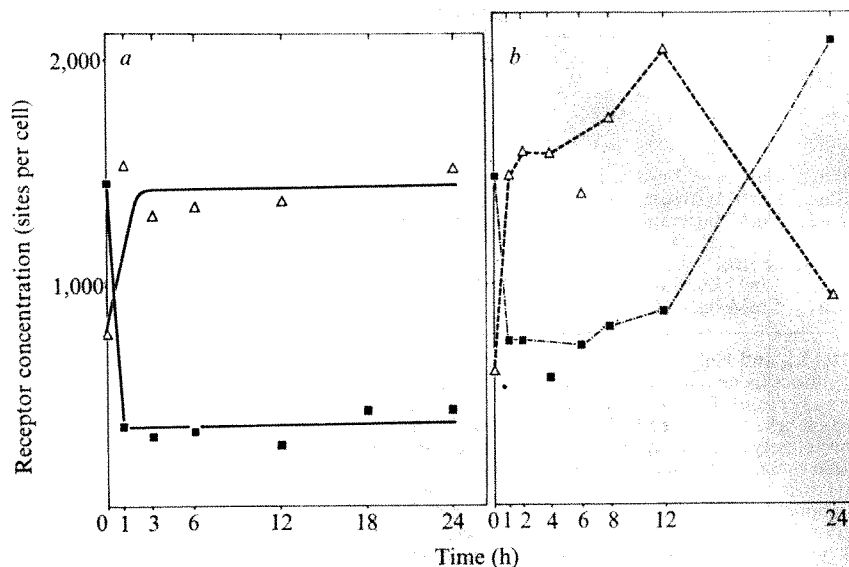


Fig. 2 The effect of a single injection of (a) tamoxifen (10 mg kg^{-1}) or (b) oestradiol benzoate (1 mg kg^{-1}) on the concentration of cytoplasmic (squares) and nuclear (triangles) oestrogen receptor sites during the 24 h following injection.



replenishment of cytoplasmic receptor. In contrast to the rat uterus, however, the presence of oestrogen receptor in the nuclear compartment of chick oviduct cells was not accompanied by any measurable oestrogenic response.

Oestradiol benzoate caused a similar degree of translocation of cytoplasmic receptor to the nucleus during the first hour (Fig. 2b), but nuclear receptor was gradually depleted after 12 h and had fallen to approximately 140% of control values by 24 h. Cytoplasmic receptor levels were replenished and had reached 150% of control by 24 h. At this time, total cellular oestrogen receptor levels had increased to about 160% of their pre-incubation level indicating oestrogen-induced synthesis of new oestrogen receptor. This pattern of receptor subcellular redistribution and synthesis was accompanied by a significant hormonal response (Fig. 1).

These results indicate that a number of currently held theories of the mechanism of action of anti-oestrogenic compounds require re-evaluation. The anti-oestrogenic activity of all non-steroidal anti-oestrogens of the triphenyl-ethylene type cannot be explained simply by their inability to stimulate cytoplasmic receptor replenishment as has been suggested previously¹². Our results show that tamoxifen in the chick oviduct is anti-oestrogenic after a single injection when cytoplasmic receptor levels are not a limiting factor in the amount of receptor translocated to the nuclear compartment. In addition the receptor which was translocated from the cytoplasm to the nucleus was unable to initiate any measurable oestrogenic response. This observation implies that the mere presence of high concentrations of oestrogen receptor in the nuclear compartment is not sufficient for hormone action and suggests, as theoretically more satisfactory, that the ligand plays an important role in determining the final activity of the ligand-nuclear receptor complex. Moreover, these results add support to the theory that there are different populations of nuclear 'acceptor' sites, some of which are biologically inactive^{1,19}. Our data, however, do not support the hypothesis²⁰ that KCl-resistant nuclear sites²¹ designate the specific 'acceptor' sites involved in the expression of maximal oestrogen-induced growth since we were unable to demonstrate any difference between oestradiol benzoate and tamoxifen treated animals with respect to the proportion of nuclear receptor sites which could be extracted with 0.5 M NaCl (manuscript in preparation).

Finally, since tamoxifen, like nafoxidine and clomiphene, is an anti-oestrogen and a weak oestrogen when tested in rodents^{22,23}, some of the discrepancies between the present data and those reported previously for rat uterine system

may be due to species differences in the mechanism of action of these compounds.

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Induction of human follicle-stimulating hormone in HeLa cells by sodium butyrate

THE ectopic production of polypeptide hormones by tumours derived from tissues that normally do not synthesise these proteins provides a means for investigating regulation of gene expression. All of the hormones ectopically synthesised by non-endocrine tumours are polypeptides or prostaglandins; however, pituitary gonadotropin production by neoplasm as opposed to chorionic gonadotropins is apparently very rare¹. Only one patient has been reported in which a bronchiogenic carcinoma produced follicle-stimulating hormone (FSH) and luteinising hormone (LH)

Table 1 Enhancement of FSH production by butyrate in HeLa cells

Subline	β -subunit of FSH antiserum FSH(ng) secreted* by 10^6 cells			Complete FSH antiserum FSH(mIU) $\dagger$ secreted* by 10^6 cells		
	Mean	Range	Proportional increase	Mean	Range	Proportional increase
HeLa ₆₅ , control	2.9	0.3–4.8	1	1.7	1.3–2.1	1
HeLa ₆₅ , 1 mM butyrate	151.7	106.1–235.8	52	6.0	5.2–6.7	3.5
HeLa ₆₅ , 5 mM butyrate	1,007.6	590.9–1590.9	347	51.0	47.7–54.5	30
HeLa ₇₁ , control	116.6	65.9–204.5	1	2.7	2.5–2.8	1
HeLa ₇₁ , 1 mM butyrate	731.7	609.8–975.6	6	16.0	14.6–17.1	6
HeLa ₇₁ , 5 mM butyrate	17,555.5	9,333.3–28,333.3	151	313.0	293.3–333.3	116

HeLa₆₅ and HeLa₇₁ cells were cultured with and without sodium butyrate for 68 h in Waymouth's medium with 10% foetal calf serum and antibiotics (penicillin 30 μ g; streptomycin 50 μ g and kanamycin 30 μ g ml⁻¹). Methods of subculturing, collecting and preparing cells for assays were as before⁴. The media and ultrasonicates of cells were tested for FSH by radioimmunoassay (RIA) using ¹²⁵I-FSH as the tracer antigen^{8–10}. Radiolabelled iodinated FSH was prepared by the Chloramine-T method and purified on polyacrylamide gel. To minimise cross-reactions with other closely related glycopolypeptides, one antiserum was raised in rabbits against the β -subunit of FSH extracted and purified from fresh frozen human pituitary glands (from Diagnostic Biochem). A second antiserum was prepared against highly purified complete human pituitary FSH (Institute of Bioendocrinology, Montreal). Radioactive antigen-antibody complexes were isolated by double-antibody precipitation technique⁸ using as the second precipitant antibody, an anti-rabbit γ globulin prepared in goats with antiserum against the β subunit and an anti-rabbit γ globulin prepared in sheep with antiserum against complete FSH. Radiolabelled precipitate was collected by centrifugation at 6,000g at 2°C and counted in a Beckman Biogamma II spectrometer. The percentage of binding of radioactive ¹²⁵I-FSH by antiserum against the β subunit of FSH was plotted against pg concentrations of purified human pituitary FSH as the standard on logit-log graph paper. The limit of sensitivity of the assay was 50 pg. The percentage binding of radioactive ¹²⁵I-FSH by antiserum against complete FSH was plotted against mIU of Second International Reference Preparation of Human Menopausal urine Gonadotropin (2nd IRP HMG) as the standard. The limit of sensitivity of the assay was 0.16 mIU. Concentrations in unknown samples were determined either from the linear standard logit-log plots or from sigmoid calibration curves on semi-logarithmic scales. Apparent differences in concentrations of FSH as measured by the two antisera are probably the result of differences in the standards used and the higher sensitivity of the radioimmunoassay with β -FSH antiserum. The results presented in the table are means of duplicate assays on three separate experiments carried out at different times. A standard of authentic FSH at seven different dilutions and a sample of human serum with a known concentration of FSH were assayed concurrently with unknown samples to ensure accuracy and reproducibility of the technique. As measured by RIA, TSH and human placental lactogen (HPL) could not be detected nor induced by sodium butyrate in these HeLa sublines.

*As measured in the medium.

$\dagger$ 1 mIU equivalent to 35ng of LER 907 FSH standard (NIH).

(ref. 2). Glycopeptide hormones—FSH, LH, thyroid-stimulating hormone (TSH) and human chorionic gonadotropin (HCG)—are composed of two dissimilar subunits: an α subunit that is common to all the glycopeptide gonadotropic hormones and a β subunit unique for each hormone that confers biological specificity³. It has been shown that HeLa cells, derived over 25 yr ago from a carcinoma of the cervix, secrete HCG (refs 4,5) and the free α subunit of glycopeptide hormones⁶. Synthesis of HCG (ref. 4) and the α subunit⁵ is markedly increased by growth in medium with sodium butyrate. We report that HeLa cells also produce pituitary FSH which is induced by butyrate. The surprising synthesis of FSH by a non-pituitary cell has required validation of the radioimmunoassay (RIA) to exclude cross-reaction with other gonadotropic hormones.

Two different antisera were used; one was prepared against highly purified human pituitary β subunit of FSH and the other against the complete hormone. The anti- β FSH serum had the following cross-reactivities: FSH 100%, HCG <1%, LH <5%, TSH <2% and growth hormone (GH) <1%. A second antiserum prepared against intact human pituitary FSH had the following cross-reactivities: <0.01% human serum albumin; <2% TSH and <0.01% with LH, HCG and GH. The tracer antigen used in the

radioimmunoassay was highly purified human pituitary ¹²⁵I labelled FSH.

Table 1 shows that two well-characterised clonal lines of HeLa cells, HeLa₆₅ and HeLa₇₁ (ref. 7), produce different basal levels of FSH. Similar differences between HeLa sublines have been observed with α chain^{5,6} and HCG production⁷ and may reflect functional alterations related to the well-known karyotypic differences between these sublines. Growth of replicate cultures of HeLa₆₅ and HeLa₇₁ cells with 1 and 5 mM butyrate caused a marked increase in FSH production (Table 1). HeLa₇₁ cells produce much more FSH in the basal and butyrate-induced state than HeLa₆₅. Dilution curves for immunoreactive substances in HeLa cell media were similar to those of authentic FSH. As shown in Table 1, the correlation of FSH values obtained by β -FSH and complete FSH assays are reasonably close except for HeLa₆₅ control where low concentrations of FSH may affect the accuracy of the assay. Differences in the apparent concentration of FSH as measured by two different antisera are probably due to the characteristics of the standards and to the greater sensitivity of β -FSH assay. Table 2 compares intracellular FSH with that secreted into the medium during 48 h of growth in medium without and with sodium butyrate. Approximately 90% of

Table 2 Comparison of intra- and extracellular levels of FSH in HeLa cells grown without and with sodium butyrate

Subline	FSH levels			
	Intracellular ng per 10^6 cells	Proportional increase	Extracellular ng per 10^6 cells	Proportional increase
HeLa ₆₅ , control, 48 h	0.2	1	4.6	1
HeLa ₆₅ , 5mM butyrate, 48 h	3.8	19	47.1	10.2
HeLa ₇₁ , control, 48 h	12.2	1	391.3	1
HeLa ₇₁ , 5mM butyrate, 48 h	66.7	5.5	1,666.6	4.3

FSH was measured by RIA as described in Table 1. The values are means of quadruplicates that varied by less than 15%.

Table 3 Induction of FSH in HeLa₆₅ cells by hydroxyurea and its synergism with sodium butyrate

Concentration of hydroxyurea (mM)	FSH levels in HeLa ₆₅ cultures			
	Grown for 96 h without butyrate		Grown for 96 h with 1 mM butyrate	
	ng per 10 ⁶ cells	Proportional increase	ng per 10 ⁶ cells	Proportional increase
0.00	23.8	1.0	69.7	2.9
0.01	33.1	1.4	115.1	4.8
0.05	36.0	1.5	125.0	5.3
0.25	80.8	3.4	310.3	13.0
1.00	416.7	17.5	736.8	31.0

FSH secreted in medium was determined by RIA as described in Table 1. The values are means of duplicates that varied by less than 10%.

the hormone synthesised is secreted into medium. Differences in the base-level production of FSH and the proportional increase mediated by butyrate as shown in Tables 1, 2 and 3, may be related to confluence of the culture and to the duration of growth with inducer.

Sodium butyrate has been shown to inhibit DNA synthesis in HeLa₆₅ cells¹¹. Recent studies have shown that other well-characterised inhibitors of DNA synthesis that are used as anti-neoplastic agents—hydroxyurea, methotrexate, and 1- β -D-arabinofuranosylcytosine—also increase HCG synthesis¹¹. For these reasons hydroxyurea which is a specific inhibitor of DNA but not RNA synthesis in HeLa₆₅ cells was studied for its capacity to induce FSH synthesis in HeLa₆₅ cells and to potentiate the effects of sodium butyrate (Table 3). Hydroxyurea stimulated FSH production and was synergistic to sodium butyrate (Table 3). Neither butyrate nor hydroxyurea when added to the radioimmunoassay affected measurements of FSH.

HeLa cells produce several proteins normally found in trophoblasts; these include the placental forms of alkaline phosphatase^{12,13}, ferritin^{14,15}, aldolase¹⁶, α -chains of glycopeptide hormones^{3,6}, and HCG^{4,5}. The availability of specific antisera against the human pituitary β subunit and complete FSH permitted measurement of this glycopeptide in the presence of excess α chains and HCG. The production of a presumptive pituitary gonadotropin by a non-pituitary cell is unexpected and suggests that the genome of HeLa cells is derepressed for highly differentiated gene products as well as oncofetal proteins. Butyrate, certain other aliphatic monocarboxylates and some antineoplastic drugs that inhibit DNA but not RNA synthesis, increase the activity of oncofetal alkaline phosphatase¹⁷ and HCG synthesis^{4,11}. Our finding that butyrate and hydroxyurea also stimulate FSH production suggests that inhibition of DNA synthesis or cell multiplication may have a role in modulating gene expression. Little is known about the mechanism by which specialised synthetic programmes are coupled to inhibition of DNA synthesis. The acquisition of a differentiated phenotype, however, is preceded by inhibition of cell division in several embryonic tissues^{18,19}. Studies on regulation of glycopeptide hormone production in HeLa cells may help elucidate aspects of gene expression in normal as well as malignant cells.

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Parallel expression of new idiotypes on T and B cells

THE structural nature of the antigen receptor on thymus-derived (T) cells is as yet unresolved^{1–4}. It has been clearly demonstrated that antigen receptors on bone marrow-derived (B) cells are immunoglobulin (Ig) molecules which are similar to the antibodies secreted by their progeny^{5–7}. This is further reflected in the presence of common variable region determinants present on Ig receptors and on antibodies produced by B cells belonging to the same clone^{8,9}. These variable region determinants are called idiotypes¹⁰. We report here the use of anti-idiotypic antibodies to determine whether receptors on T and B cells specific for the hapten phosphorylcholine (PC) share idiotypic determinants. The majority of PC-specific B cells in BALB/c mice have receptors bearing an idiotypic characteristic of the myeloma protein TEPC 15 (T15) (ref. 8). To determine whether receptors on PC-specific T cells also reflect this 'idiotypic homogeneity', we have induced PC-specific helper T cells and attempted to inhibit their activity using anti-T15 antibodies. Our results indicate that receptors on PC-specific T and B cells share similar idiotypes.

To obtain hapten-specific T cells, we immunised BALB/c mice with either PC-conjugated MOPC 315 (PC₃-M315) (ref. 11) or dinitrophenyl (DNP)-conjugated mouse immunoglobulins⁷ (DNP₉-MIg) as previously described¹². At least 6 weeks after priming, splenic T cells were purified by nylon wool filtration¹³. Bovine serum albumin (BSA)-primed B cells were obtained from BALB/c mice immunised as previously described¹². At least 6 weeks after priming, spleen cells from these mice were treated with anti-Thy 1.2 serum and complement.

The PC-M315 and DNP-MIg-primed splenic T cell populations were assayed for helper activity in an adoptive transfer system with BSA-primed B cells. Either 50 μ g of aqueous PC-conjugated BSA (PC₂₈-BSA) (ref. 11) or 50 μ g of aqueous DNP-conjugated BSA (DNP₄₅-BSA) (ref. 7) was given intravenously with the cell inoculum. The mice

Table 1 Demonstration of PC- and DNP-specific helper T cells

BSA-primed† B cells	No. of cells transferred ($\times 10^6$)* PC-primed‡ T cells	DNP-primed T cells	Antigen	Indirect anti-BSA§ PFC per spleen ($\times 10^3$)
10	—	—	PC ₂₈ -BSA	14 (11-18)
—	15	—	PC ₂₈ -BSA	2 (1-3)
—	—	15	PC ₂₈ -BSA	0.5 (0.4-0.6)
10	—	—	DNP ₄₅ -BSA	13 (10-17)
—	15	—	DNP ₄₅ -BSA	2 (1-3)
—	—	15	DNP ₄₅ -BSA	0.5 (0.3-0.6)
10	15	—	PC ₂₈ -BSA	297 (266-331)
10	15	—	DNP ₄₅ -BSA	33 (25-43)
10	—	15	DNP ₄₅ -BSA	185 (163-210)
10	—	15	PC ₂₈ -BSA	36 (31-42)

*Cells were transferred into 600 R irradiated BALB/c mice. Animals received 50 μ g of aqueous antigen simultaneously with the cells and were killed on day 8 post cell transfer.

†Spleen cells from BSA-primed mice were treated with anti-Thy 1.2 antiserum and complement.

‡Mice were primed with either PC₂₈-M315 or DNP₄₅-MIg. T cells were purified by nylon wool filtration¹³. Both T cell populations contained > 90% T cells and 1% Ig bearing cells as assessed by immunofluorescent staining.

§Geometric mean of the responses of four or five animals per group. Numbers in parenthesis represent one standard error about the mean. The direct anti-BSA PFC response in all groups was less than 5% of the total response. Indirect anti-BSA PFC were developed using a polyvalent rabbit anti-mouse immunoglobulin antiserum.

were killed 8 d later and their spleens assayed for anti-BSA plaque-forming cells¹⁴ (PFC) using BSA-conjugated sheep erythrocytes¹⁵ as indicator cells.

Transfer of 15×10^6 PC-primed T cells together with 10×10^6 BSA-primed B cells and PC₂₈-BSA, resulted in a response of 29,700 indirect anti-BSA PFC per spleen (Table 1). This response is 19-fold higher than the background response calculated by summing the responses obtained on transfer of the same cell populations separately, that is, 1,600 anti-BSA PFC. When the same cell populations were transferred with DNP₄₅-BSA as antigen, the resulting anti-BSA PFC response was only two-fold above background (Table 1). Similarly, transfer of 15×10^6 DNP-primed T cells with 10×10^6 BSA-primed B cells resulted in an anti-BSA PFC response 12-fold above background with DNP₄₅-BSA as antigen and only two-fold higher than background when PC₂₈-BSA was used as antigen (Table 1).

To assess whether the helper activity of PC-specific T cells could be inhibited with anti-T15 antibodies, irradiated BALB/c mice were injected with anti-T15 antibodies 24 h before and at the time of, transfer of PC-primed T cells and BSA-primed B cells. Antigen (50 μ g of PC₂₈-BSA) was given 16 h after cell transfer. The animals were killed 8 d after antigen administration and their spleens assayed for anti-BSA PFC.

The data in Table 2 show that anti-T15 antibodies com-

pletely inhibit PC-specific helper activity. When 10×10^6 PC-primed T cells were transferred with 5×10^6 BSA-primed B cells and PC₂₈-BSA into conventional irradiated recipients, the resulting anti-BSA PFC response was six-fold higher than background, that is, 9,100 against 1,500 anti-BSA PFC per spleen respectively. Treatment of the recipients with anti-T15 antibodies inhibited the anti-BSA PFC response to less than 1% of the control response, that is, 1,400 anti-BSA PFC per spleen. The specificity of inhibition was established by assessing the effect of anti-T15 antibodies on the expression of DNP-specific helper activity. When 10×10^6 DNP-primed T cells were transferred with 5×10^6 BSA-primed B cells and DNP₄₅-BSA, the resulting anti-BSA PFC response was sevenfold above background, that is, 8,600 against 1,200 anti-BSA PFC spleen. Treatment of DNP-primed T cell recipients with anti-T15 antibodies did not significantly affect the anti-BSA PFC response which remained ninefold higher than background (Table 2).

The irradiated recipients used in these experiments contained high levels of T15-bearing Ig with demonstrable anti-PC activity¹⁶. It is conceivable that on transfer into irradiated recipients, PC-specific T cells bind PC₂₈-BSA which in turn picks up circulating T15 positive anti-PC antibodies. Thus, PC-specific T cells obtained from conventional BALB/c mice could bear T15-negative receptors and their helper activity might still be inhibited by anti-T15

Table 2 Inhibition of PC-specific helper activity with anti-T15 antibodies

BSA-primed† B cells	No. of cells transferred ($\times 10^6$)* PC-primed‡ T cells	DNP-primed T cells	Antigen	Anti-T15§	Indirect anti-BSA PFC per spleen ($\times 10^3$)¶	% of control**
5	—	—	PC ₂₈ -BSA	—	8 (6-9)	—
—	10	—	PC ₂₈ -BSA	—	7 (4-12)	—
5	10	—	PC ₂₈ -BSA	—	91 (74-112)	100
5	10	—	PC ₂₈ -BSA	+	14 (10-19)	< 1
5	—	—	DNP ₄₅ -BSA	—	9 (7-14)	—
—	—	10	DNP ₄₅ -BSA	—	3 (2-4)	—
5	—	10	DNP ₄₅ -BSA	—	86 (76-97)	100
5	—	10	DNP ₄₅ -BSA	+	106 (90-125)	127

*Cells were transferred intravenously into 350 R irradiated BALB/c mice. Animals received 50 μ g of aqueous antigen intravenously, 16 h after cell transfer, and were killed on day 8 post antigen administration.

†See legend for Table 1.

‡See legend for Table 1.

§0.2 ml of a 1/2 dilution of A/J anti-T15 antiserum was administered intraperitoneally 16 h prior to, and at the time of, cell transfer. The idiotype specificity of this antiserum has been reported elsewhere^{17,18}.

¶Geometric means of the responses of three to six animals per group (all groups with T+B cells contained six animals). The direct anti-BSA PFC response in all groups was less than 5% of the total response. Indirect anti-BSA PFC were developed using a polyvalent rabbit anti-mouse immunoglobulin antiserum.

**% of control response was calculated as follows: Inhibition of PC-specific helper activity: $[14 - (8 + 7)]/[91 - (8 + 7)]$ where (8 + 7) is the background anti-BSA PFC response obtained on transfer of T or B cells alone with PC₂₈-BSA. Similarly, inhibition of DNP-specific helper activity $[106 - (9 + 3)]/[86 - (9 + 3)]$.

Table 3 Failure of anti-T15 antibodies to inhibit PC-specific helper activity induced in T15 negative BALB/c mice

BSA-primed† B cells	No. of cells transferred ($\times 10^6$)*		Antigen	Anti-T15§	Indirect anti-BSA¶ PFC per spleen ($\times 10^3$)		% of control**
	PC-primed T cell†† T15+	T15-					
5	—	—	PC ₂₈ -BSA	—	28 (25-31)	—	—
—	7.5	—	PC ₂₈ -BSA	—	2 (1.9-2.3)	—	—
5	7.5	—	PC ₂₈ -BSA	—	168 (141-200)	100	100
5	7.5	—	PC ₂₈ -BSA	+	18 (15-21)	< 0.7 ^a	< 0.7 ^a
5	3.8	—	PC ₂₈ -BSA	—	133 (116-154)	100	100
5	3.8	—	PC ₂₈ -BSA	+	31 (24-39)	1 ^b	1 ^b
5	—	—	PC ₂₈ -BSA	—	13 (10-17)	—	—
—	—	10	PC ₂₈ -BSA	—	2 (1.5-2.8)	—	—
5	—	10	PC ₂₈ -BSA	—	94 (78-113)	100	100
5	—	10	PC ₂₈ -BSA	+	111 (92-124)	122 ^c	122 ^c

*See legend for Table 2.

†See legend for Table 1.

‡Splenic T cells were isolated from either conventional T15-positive (T15⁺) BALB/c mice which contained 40-80 $\mu\text{g ml}^{-1}$ of circulating Ig bearing the T15 idiotype or from T15-negative (T15⁻) BALB/c mice which had been neonatally suppressed with 0.1 ml of A/J anti-T15 antiserum and contained < 2.5 $\mu\text{g ml}^{-1}$ of circulating Ig bearing the T15 idiotype. Both types of donor were primed with PC₂₈-M315. In both cases isolation of splenic T cells resulted in lymphocyte populations which contained > 90% T cells and 1% Ig bearing cells as assessed by immunofluorescence staining.

§See legend for Table 2.

¶See legend for Table 2.

**See legend for Table 2 for explanation of calculations: a, $(18-30)/(168-30) = < 1/138 = 0.7\%$; b, $(31-30)/(133-30) = 1/103 = 1\%$; c, $(111-15)/(94-15) = 122\%$.

antibodies.

To test this possibility we took advantage of the observation that receptors on PC-specific precursor B cells in BALB/c mice neonatally suppressed with anti-T15 antibodies bear T15-negative idiotypes^{17,18}. If neonatal idiotype suppression induces a parallel change in receptors expressed by PC-specific T cells, their helper activity should not be inhibited by anti-T15 antibodies, unless they adsorb T15-positive anti-PC antibodies naturally present in the conventional BALB/c recipients. The results in Table 3 show that when either 7.5×10^6 or 3.8×10^6 PC-primed T cells from T15-positive donors were transferred with 5×10^6 BSA-primed B cells, the resulting anti-BSA PFC responses obtained were sixfold higher than background. Again, these responses were completely abolished by treatment of recipients with anti-T15 antibodies, that is, less than 1% of the control response (Table 3). Comparable helper activity was obtained on transfer of 10×10^6 PC-primed T cells from T15-negative donors. Treatment of recipients with anti-T15 antibodies, however, did not significantly affect the anti-BSA PFC response which remained sevenfold above background, that is, 122% of the control response (Table 3). We conclude that the target of anti-T15 antibodies is not a molecule passively adsorbed on the T cell surface either directly or through antigen bridge; it is the functional receptor present on PC-specific T cells which bears the T15 idiotype.

These studies demonstrate a parallel behaviour between antigen receptors on PC-specific T and B cells. As has been previously shown, antibodies directed towards the T15 idiotype specifically inhibit the function of PC-specific B cells from T15-positive BALB/c mice⁸. Similarly, the helper activity of PC-specific T cells from T15 positive mice is also inhibited by anti-T15 antibodies. Furthermore, PC-specific T and B cells from neonatally suppressed mice bear receptors which lack the T15 idiotype. These results suggest (1) that the T15 idiotype is present in the repertoire of PC-specific T and B cells and (2) that neonatal suppression of this idiotype induces the expression of T15-negative receptors in both lymphocyte lineages.

Consistent with these findings is the recent work of Binz and Wigzell which demonstrated that anti-idiotypic antibodies prepared against alloantibodies specifically removed the reactivity of T cells to the same alloantigen¹⁹. In addition, Eichmann and Rajewsky²⁰ and Black *et al.*²¹ demonstrated that anti-idiotypic antibodies were able to

induce priming of both B and T cells with specificity for the same antigen. In addition to the present report, these results strongly suggest that the specificity of antigen recognition by B and T cells is very similar and that this specificity is imparted by structurally similar molecules. It has been reported, however, that T cells contain and secrete antigen-specific factors bearing markers which are *Ir* gene products^{22,23}. In addition, products of the major histocompatibility complex restrict the function of T lymphocytes of any specificity²⁴⁻²⁷. It is possible, therefore, that the V-region structures of receptors on T and B cells specific for the same antigen, bear similar idiotypes associated with structurally different molecules.

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Failure to demonstrate allotype-specific helper cells for antibody production in the rat

THE way in which the immune response is regulated is poorly understood but network theories, based on idio-anti-idiotypic interactions have been widely discussed¹. It has been suggested^{2,3} that in addition to the idiotypic-based network an allotype network also exists. This hypothesis rests on the demonstration³ that in a particular mouse strain, carrier-primed T cells from Ig 1-a homozygotes collaborated in a secondary anti-DNP (dinitrophenol) response with Ig 1-a allotype, but not Ig 1-b allotype, B cells from DNP-primed 1-a/1-b heterozygotes. In attempting to understand the regulation of the immune response it is clearly essential to establish whether the heavy chain allotype specificity of helper T cells shown by these experiments is common to all allotypes and is not a particular feature of the system studied. Here we describe experiments in which we were unable to demonstrate allotype-specific helper cells for antibody production in the rat.

T cells and DNP/BGG were injected intravenously into irradiated PVG/c homozygotes and the number of 1-a allotype anti-DNP plaque-forming cells per recipient spleen was determined using an autoradiographic plaque assay⁷.

The results show that in the absence of helper T cells hapten-primed B cells on transfer to irradiated recipients respond poorly to challenge with the priming antigen (Table 1). In contrast a greatly augmented B cell response is obtained when carrier-primed T cells are provided even if these derive from donors of the opposite light chain allotype. (The variation between the magnitude of augmentation in the three experiments is not statistically significant being largely a reflection of the wide variation in the small response produced by the purified B cell populations transferred.) Thus the data give no indication of allotype specificity of T cell help in this system. Because the light chain allotype is present on all classes of rat immunoglobulin the results do not exclude the possibility that allotype-specific helper cells exist for some classes but not others. Nor is it possible to rule out the existence of a cell type that regulates the expression of the two light chain allotypes in 1-a/1-b heterozygotes. It is clear, however, that in the rat carrier-primed T cells and hapten-primed B

Table 1 Collaboration between T cells from 1-b homozygote donors and 1-a allotype B cells

Experiment no.	TDL transferred $\times 10^{-6}$		No. of 1-b/1-b recipients†	1-a anti-DNP PFC per spleen (mean and range)	Collaborative increase§
	T cells* (1-b/1-b) 7.4	B† cells 0			
1	0	2.9 (1-a/1-b)	2	713 (285-1,140)	39
	7.4	2.9 (1-a/1-b)	2	28,070 (27,640-28,500)	
	7.4	0	3	0	
2	0	2.9 (1-a/1-b)	2	171 (114-228)	131
	7.4	2.9 (1-a/1-b)	2	22,340 (18,580-26,100)	
	7.4	0	3	0	
3	0	2.7 (1-a/1-a)	2	228 (0-456)	259
	7.4	2.7 (1-a/1-a)	2	59,050 (49,470-68,620)	
	7.4	0	3	0	

*PVG/c TDL depleted of B cells from rats primed with DNP₂₉BGG 7-12 weeks previously.

†B cells selected by FACS from TDL of DNP₂₉BGG primed (PVG/1-a $\times$ PVG/c)_{F1} (first two experiments) or PVG/1-a homozygote (third experiment) rats.

‡Recipients were PVG/c rats given 500 rad (first two experiments) or 660 rad (third experiment) 6 h before cell transfer and challenge.

§Collaborative increase refers to the factor by which the 1-a PFC response is augmented by the addition of the carrier-primed T cells.

To investigate the generality of the findings of Herzenberg *et al.*³ we examined the allotype specificity of helper T cells using the rat light chain allotype marker⁴. B and T cells from appropriate donors, primed several weeks earlier with DNP-bovine γ globulin (DNP-BGG), were injected intravenously together with DNP-BGG into irradiated recipients. The splenic anti-DNP plaque-forming cell response was assayed 1 week later. Thoracic duct lymphocytes (TDL) from PVG/1-a and (PVG/1-a $\times$ PVG/c)_{F1} rats were used as a source of B cells. PVG/1-a is a new strain, congenic with PVG/c, but carrying the 1-a light chain allotype. Heterozygotes were obtained from the sixteenth backcross and homozygotes from an incross of this generation. T cells were obtained from TDL of PVG/c homozygotes. B and T cell donors were primed intraperitoneally with alum-precipitated DNP-BGG and 2×10^6 Pertussis organisms 6-12 weeks before use. B cells were labelled by incubation with rabbit F(ab')₂-anti-rat Fab followed by fluorescenated horse-anti-rabbit Fab and isolated using a fluorescence-activated cell sorter⁵. T cells were purified by rosetting TDL with rabbit-anti-rat F(ab')₂ coated sheep red blood cells and subsequent fractionation on an Isopaque-Ficoll gradient⁶. Various mixtures (see Table 1) of B cells,

cells derived from donors of different allotype can collaborate efficiently.

Elson and Taylor⁸ using the same allotypic marker as Herzenberg *et al.*³, but with a different congenic strain combination, found no evidence of allotype restriction of helper T cells. These findings taken together with our own suggest that the contrary findings of Herzenberg *et al.* may be dependent in some way on the particular mouse strain used by these workers.

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T-cell mediated immunity towards antigen(s) on isologous erythrocytes

SPLEENS of normal mice contain large numbers of cells which produce antibody against antigenic determinants on their own erythrocytes^{1,2}. To demonstrate this autoimmune response, the mouse erythrocytes (MRBC) must be treated with proteolytic enzymes, such as trypsin or bromelain, in order to reveal hidden autoantigen(s)². Treatment of mice with anti-lymphocyte serum (ALS) causes a 20-fold rise in the number of cells producing antibody to bromelain-treated mouse erythrocytes (BrMRBC) (ref. 2). Based on this observation, Cunningham has proposed that the number of cells making anti-BrMRBC antibody is controlled by an active, ongoing, T-cell induced suppression³. We report here that the culturing of normal mouse spleen cells *in vitro* not only allows the development of cells producing antibody to BrMRBCs, but also T cells which mediate, on transfer, delayed-type hypersensitivity (DTH).

Normal C57B1/6J (6–8-week-old) mouse spleen cells were cultured in Marbrook chambers⁴ at a concentration of $1.5 \times 10^7 \text{ ml}^{-1}$ in CMRL 1066 medium, supplemented with 10 ml l^{-1} each of 100-times concentrated MEM non-essential amino acids, L-glutamine, sodium pyruvate and 17% heat-inactivated foetal calf serum. The preparation of BrMRBC and the plaque-forming cell (PFC) assays were carried out as described by Cunningham¹. The separation of immunoglobulin-bearing cells, the transfer of cells into the footpads of mice, and the measurement of DTH reactions have been described in detail elsewhere^{5,6}.

When suspensions of normal spleen cells are cultured *in vitro*, a substantial anti-BrMRBC PFC response develops within 2 d (Table 1). The cultured spleen cells also induce DTH reactions when they are injected with BrMRBC into the footpads of normal mice (Table 1). In contrast to the antibody response, which peaks at day 2, the largest DTH reactions are obtained with cells taken after 4 d in culture and at a time when the antibody response is declining.

The T-cell dependence of the DTH reaction was demonstrated by treating cultured spleen cells with anti-Thy 1.2 serum and complement. Whereas untreated cultured spleen cells or cells treated with normal serum and complement induced large DTH reactions, cells treated with anti-Thy 1.2 serum and complement produced only a background footpad swelling (Table 2). Furthermore, depleting cultured

Table 1 Development of T cells which mediate delayed-type hypersensitivity against BrMRBC

Days in culture	DTH reactions against BrMRBC*	Plaque-forming cells against BrMRBC†
0	$4.2 \pm 0.21^\dagger$	154 ± 54
1	5.5 ± 0.23	960 ± 120
2	10.1 ± 0.95	$8,620 \pm 710$
3	14.8 ± 0.49	$6,640 \pm 509$
4	19.5 ± 0.6	$2,010 \pm 346$

*Cultured spleen cells were collected, washed and the red cells and dead cells removed by centrifugation on Isopaque/Ficoll¹⁸. 10^7 recovered viable cells with 10^8 BrMRBC were injected in volumes of $15 \mu\text{l}$ into the footpads of normal mice. The increase in footpad swelling is expressed in units of $0.01 \text{ mm} \pm \text{s.e.}$

†Background footpad swellings ranging between 3.5 and 4.5 were caused by the trauma of injection.

‡Plaques per 10^7 viable recovered cells $\pm \text{s.e.}$

Table 2 T-cell dependence of the delayed-type hypersensitivity reaction against BrMRBC

Treatment of cultured spleen cells*	BrMRBC added	DTH reaction
None	+	17.0 ± 0.15
None	—	4.3 ± 0.21
Anti-Thy 1.2 + C	+	5.0 ± 0.22
Normal serum + C	+	17.5 ± 1.7
Depletion of Ig ⁺ cells	+	18.0 ± 0.8

*Spleen cells were cultured for 4 d, collected, washed and the red cells and dead cells removed by centrifugation on Isopaque/Ficoll¹⁸. Depletion of immunoglobulin-bearing cells and treatment with anti-Thy 1.2 serum are described in detail elsewhere^{5,6}. The efficiency of depleting Ig-bearing cells has been shown⁶ to be $> 97\%$. 10^7 cultured spleen cells with 10^8 BrMRBC were injected in volumes of $15 \mu\text{l}$ into the footpads of normal mice. The increase in footpad swelling is expressed in units of $0.01 \text{ mm} \pm \text{s.e.}$ Background footpad swellings ranging of 3.5–4.5 were caused by the trauma of injection.

spleen cells of immunoglobulin-bearing cells before cell transfer did not affect the level of DTH obtained (Table 2). The specificity of the DTH reactions was studied by transferring, into the footpads of normal mice, mixtures of cultured cells and various antigens (Table 3). Only with BrMRBC, sheep red blood cells (SRBC) or bromelain-treated SRBC, but not with several other antigens tested, including untreated MRBC (Table 3), were DTH reactions elicited BrMRBC and SRBC also cross react at the antibody level⁷.

These findings raise the question of how this cell-mediated autoimmune response is maintained *in vivo* at a relatively low level. Cunningham³ has proposed that suppressor T cells control the number of PFC against BrMRBC. A similar mechanism may also control the T-cell mediated immunity (CMI). This view is supported by our findings that suppressor T cells are induced with the establishment

Table 3 Specificity of the delayed-type hypersensitivity reaction induced by cultured spleen cells

Antigen	Cultured cells†	DTH reaction
None	+	4.1 ± 0.13
BrMRBC*	+	18.5 ± 1.2
BrMRBC	—	4.2 ± 0.2
MRBC	+	4.16 ± 0.13
SRBC	+	15.1 ± 0.59
BrSRBC*	+	15.5 ± 1.06
HRBC	+	4.3 ± 0.54
BrHRBC*	+	6.3 ± 0.72
GRBC	+	4.1 ± 0.54
BrGRBC*	+	4.2 ± 0.72
KLH‡	+	3.5 ± 0.23

*Bromelain-treated mouse (M), sheep (S), horse (H) or goose (G) erythrocytes.

†Spleen cells were cultured for 4 d, collected, washed and the red cells and dead cells removed by centrifugation on Isopaque/Ficoll. 10^7 recovered viable cells with antigen were injected in volumes of $15 \mu\text{l}$ into the footpads of normal mice. DTH reactions were read 24 h later. Additional experimental details as for Table 1.

‡ $50 \mu\text{g}$ Keyhole limpet haemocyanin (KLH) was injected with cultured spleen cells.

of different, and opposing, types of immunity. Thus, T cells induced under conditions of humoral immunity can suppress CMI responses⁸, whilst CMI cells can repress the induction of humoral immunity⁹. BrMRBC antigens may induce a balanced state of CMI and IgM antibody with different populations of suppressor T cells controlling each type of immunity. If these postulates are correct, then manipulations which suppress CMI should also enhance the humoral immune response to BrMRBC. Thus, for

example, administration of ALS, which is known to selectively suppress CMI in sensitised animals¹⁰, also causes an increase in the number of PFC to BrMRBC².

Numerous other studies have shown that a wide range of self-antigens exist which are hidden on intact cells¹¹⁻¹³. Degenerating or dead cells may reveal these cryptic antigens which then stimulate both humoral and CMI responses. This autoimmunity seems harmless and may, in fact, be beneficial in removing dying or dead cells.

Furthermore, it is possible that such autoimmune responses may act as an immune surveillance mechanism that prevents the development of 'abnormal' cells such as tumour cells. Normal cells would be safe from immune attack because the self-antigens are hidden. An 'abnormal' cell that has an altered cell membrane may, however, have these cryptic sites exposed resulting in their immune destruction. Such an immune surveillance mechanism has the advantage that the immune response is primed and therefore need not adapt to new antigens on the tumour cells. Many naturally-occurring antibodies^{14,15} and cytotoxic cells^{16,17} specific to spontaneous or carcinogen-induced tumours have been found in normal mice. The antigens, against which this natural immunity is directed, have not been identified. Nonetheless, the wide-ranging differences that are known to exist between normal and tumour cell membranes are compatible with the view that natural immunity may be directed against normally hidden self-antigens that have become exposed on the tumour cells.

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Uptake of nucleosides in density-inhibited cultures of 3T3 cells

AN increase in the rate of nucleoside incorporation into acid-soluble pools is one of the earliest events seen when fibroblasts arrested in the G₁/G₀ or A state¹ are stimulated to grow by the addition of serum²⁻⁵. The stimulation of uridine uptake is preceded by a lag phase of several

minutes⁶ and is not prevented by inhibitors of protein synthesis⁷. The uptake of nucleosides by mammalian cells seems to proceed in two steps: (1) the nucleosides are transferred rapidly across the plasma membrane by a facilitated diffusion mechanism⁸ and (2) the intracellular nucleosides are phosphorylated by specific nucleoside kinases and the nucleosides formed are trapped intra-

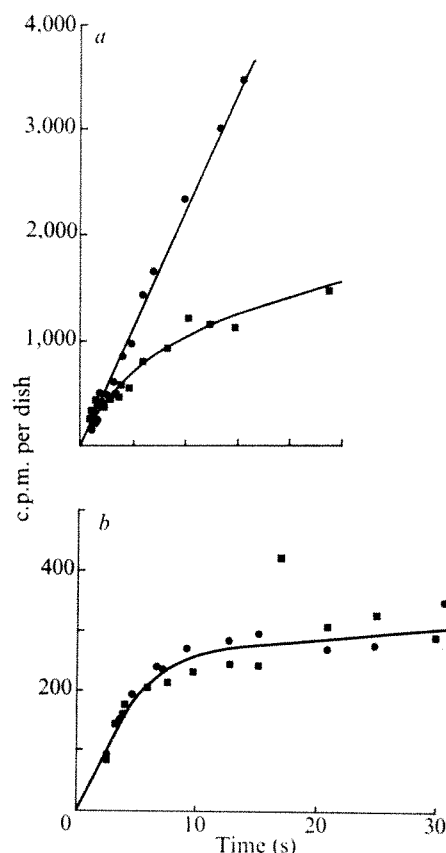


Fig. 1a, Uptake of labelled uridine by 3T3 cells as a function of time at 37 °C, in cultures previously activated by exposure to 10% foetal calf serum for 30 min, (circles) or in quiescent cells, exposed only to a serum-free medium (squares). Swiss mouse 3T3 cells¹⁴, propagated as previously described¹⁵, were subcultured into 30-mm Nunc dishes in 6% foetal calf serum; medium was changed at 3 d. Uptake activity was always assayed on confluent cells 12-15 d later. Uptake was measured by adding at zero time about 2 ml of Dulbecco modified minimal Eagles' medium (DME) containing 1.5 μM uridine, labelled radioactively as 5-³H-uridine at 1.67 Ci mmol⁻¹. At the times indicated on the abscissa, the medium was removed rapidly and the dishes were washed six times with ice-cold 0.14 M NaCl and the cultures were extracted with 1 ml of 5% trichloroacetic acid at 4 °C for 20 min. Acid-soluble pools (0.9 ml) were removed for scintillation counting with a Triton-toluene solution of phosphor. Samples for the zero-time values received the same medium and radioactivity, with the exception that it contained 1 μM NBMI, for about 2 s, before washing six times with an ice-cold solution of 1 μM NBMI in saline. The results are expressed on the ordinate as c.p.m. per dish from which the zero-time values of 281 ± 62 (s.e.m., four determinations) c.p.m. per dish for the serum-stimulated cultures, and 279 ± 55 (s.e.m., four determinations) c.p.m. per dish for the non-stimulated cultures, have been subtracted. **b**, Uptake of labelled cytosine-β-D-arabino- side as a function of time at 37 °C into 3T3 cells stimulated by serum (circles) or into non-stimulated cells (squares). Uptake was measured as for Fig. 1a, except that 5-³H-cytosine-β-D-arabino- side was at specific activity 0.5 Ci mmol⁻¹ and concentration 5 μM. The zero-time values were 89 ± 2 (s.e.m., three determinations) c.p.m. per dish for the serum-stimulated cells and 54 ± 8 (s.d. two determinations) c.p.m. per dish for the non-stimulated cultures.

cellularly⁹. Although the regulation of nucleoside uptake has been studied in some detail³⁻⁷, it is not known which of these two steps is activated by growth-promoting agents. We have investigated which step of nucleoside uptake is stimulated by serum in density-inhibited cultures of 3T3 cells. The results indicate that phosphorylation is the rate-limiting step in nucleoside uptake by quiescent cells. Growth stimulation by serum enhances the rate of phosphorylation, but apparently not that of transport.

Figure 1a shows the time course of uridine uptake by cultures that were exposed to serum for 30 min and therefore have a fully stimulated uptake⁶ compared with cultures shifted to synthetic medium alone (non-stimulated cultures). While the time course is linear in serum-activated cells, the pattern is drastically different in the non-stimulated cultures. For the first 4 or 5 s both sets display no apparent difference in uptake while afterwards the curve depicting the uptake by non-stimulated cultures levels off sharply. These results suggest that the initial rate of uridine transport is not substantially different in serum-stimulated and non-stimulated cultures, but that intracellular trapping by the kinase is much less efficient in resting cells. We also measured the time-course of uptake of cytosine- β -D-arabinoside, a nucleoside which is transported

across the cell membrane¹⁰ partly by the uridine 'carrier' system (personal communication from Z. I. Cabantchik and Y. Eilam) but is phosphorylated by deoxycytidine kinase rather than by uridine kinase^{11,12}. Figure 1b shows that the initial velocity of entry of cytosine- β -D-arabinoside into serum-stimulated cells does not differ from that of the controls. This experiment reinforces the conclusion that (nucleosides are transferred rapidly across the cell membrane of mammalian cells whether they are resting or stimulated to grow.

If the biphasic shape of the uptake curve of the non-stimulated cultures shown in Fig. 1a results from a rapid transfer of nucleoside followed by a low rate of phosphorylation, it should be possible to convert such a biphasic curve into a straight line when transport is selectively inhibited, so that transport (rather than internal phosphorylation) becomes rate-limiting. NBMI (6-nitrobenzyl mercaptinosine), a highly potent and specific inhibitor of nucleoside transport¹³, inhibits uptake

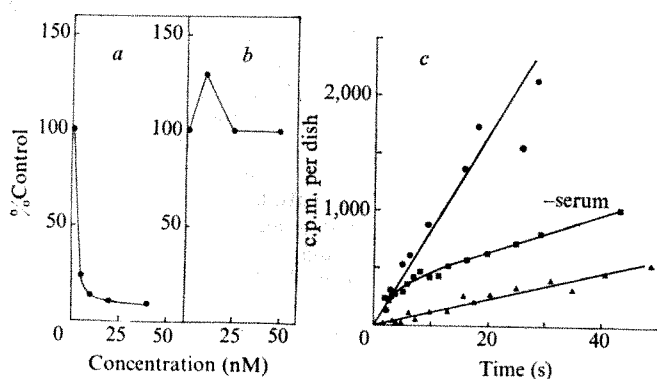


Fig. 2 *a*, Inhibition by NBMI of uridine uptake into quiescent 3T3 cells. Uridine uptake was measured as described for Fig. 1, except that the time of incubation was everywhere 6 min (at 37 °C). The cultures received NBMI at the concentration indicated on the abscissa for 10 min before the assay uptake. *b*, Effect of NBMI on the activity of uridine kinase isolated from 3T3 cells. The assay of uridine kinase was modified from that of Mizel and Wilson¹⁶. Cultures of quiescent 3T3 cells were washed with isotonic saline and scraped off the dish with a rubber policeman. The cells were pelleted, resuspended in 10 mM Tris-HCl, pH 7.5; 10 mM KCl; 2 mM MgCl₂, swollen for 10 min and disrupted in a Dounce homogeniser. The homogenate was centrifuged at 23,400g for 20 min. The incubation was performed at 37 °C in 400 μ l of solution containing 50 mM HEPES, pH 7.4; 2 mM ATP; 2 mM MgSO₄; 4 mM 3-PGA and 1 μ M 5-³H-uridine (5 Ci mmol⁻¹) and concentrations of NBMI indicated on the abscissa. The reaction was started by the addition of cell extract and stopped by boiling (3 min); samples of 100 μ l were removed at 3-min intervals from the incubation mixture. After centrifugation to remove denatured protein, 50- μ l samples were applied to DEAE filters (Whatman DE-81). The filters were washed and the radioactivity retained was measured as before¹⁶. The time course of the reaction was linear for at least 9 min of incubation. *c*, Uptake of labelled uridine as a function of time into 3T3 cells stimulated by serum (squares), or into non-stimulated cells (circles) or into non-stimulated cells treated for 30 min at 37 °C with 1.5 nM NBMI, uptake being measured in the presence of a similar concentration of NBMI (triangles). Uptake was measured as described for Fig. 1, except that the zero-time values were obtained in the presence of 50 nM NBMI, and the washing solution also contained this reduced concentration of NBMI. The zero-time values were: for the serum-stimulated cultures, 456 $\pm$ 23 (s.e.m., three determinations) c.p.m. per dish, for the non-stimulated cultures, 209 $\pm$ 50 (s.e.m., three determinations) c.p.m. per dish.

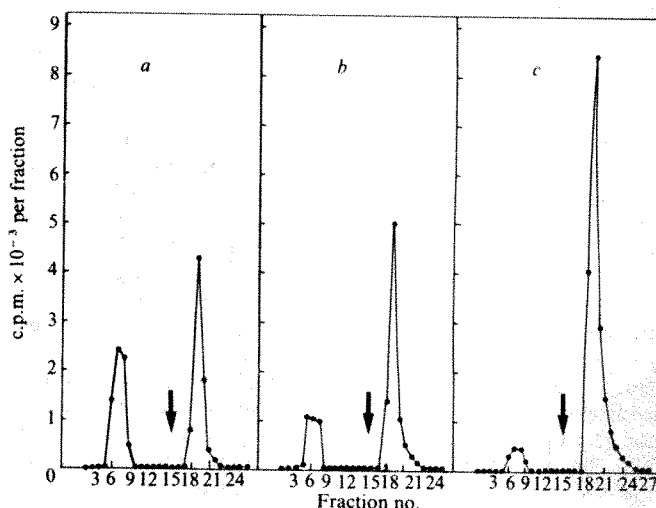


Fig. 3 Ion exchange chromatography of acid-soluble pools from quiescent cultures of 3T3 cells in different experimental conditions. Cultures of 3T3 cells were grown as described for Fig. 1 but in 90-mm diameter plastic dishes. The cultures were exposed for 30 min at 37 °C to DEM alone (*a*), DEM containing 0.5 nM NBMI (*b*) or DEM containing 10% foetal calf serum (*c*). At the end of the incubation the medium was removed rapidly, replaced by 3 ml of medium of the same composition in each case but containing 5-³H-uridine (28 Ci mmol⁻¹ 10 μ Ci ml⁻¹). After 6 s the radioactive medium was aspirated rapidly and the dishes were washed five times with 10 ml of ice-cold isotonic NaCl containing 100 nM NBMI. Then the cultures were extracted with 2 ml of 5% trichloroacetic acid for 1 h. The extracts from two cultures treated identically were combined and extracted five times with ether. (As control, we verified that 5-³H-uridine remains in the aqueous phase.) The aqueous phases were applied to columns (0.6 $\times$ 10 cm) of Bio-Rad AG1-x2 anion exchange resin (200-400 mesh) which had been converted to the formate form. Free uridine was eluted with 15 ml of H₂O; the phosphorylated nucleoside was subsequently eluted with 1 N HCl. The change to HCl is indicated by the arrows. Fractions of 1 ml were collected directly into scintillation vials; the samples 1 to 15 were adjusted to 1N HCl and then all the vials received 10 ml of Triton-toluene scintillation fluid. The radioactivity was determined in a Packard scintillation counter. To determine the amount of radioactive uridine that might remain in the extracellular space after washing, parallel cultures in DEM were exposed to 100 nM NBMI for 30 min, labelled for 3 s in the presence of the inhibitor to prevent the entry into the cells of any uridine and washed as described for the other cultures. In these conditions only a small amount of radioactivity was eluted in the tubes 5, 6, 7 and 8 (20, 66, 71 and 21 c.p.m. above background respectively) and thus, the radioactivity shown in the graphs represents only intracellular pools. The extensive washing procedure before extraction of the intracellular acid-soluble pools eliminates most of the extracellular uridine and the presence of NBMI during the washing prevents the efflux of free uridine¹⁴.

of uridine by intact 3T3 cells (Fig. 2a), but it does not alter nucleoside phosphorylation by cell-free homogenates prepared from parallel cultures (Fig. 2b). Thus, transport of nucleosides can be manipulated separately from their internal phosphorylation in 3T3 cells. When transport is made rate-limiting by addition of NBMI to quiescent cultures of 3T3 cells, the biphasic curve of uridine uptake is replaced by a straight line (Fig. 2c). When added to serum-stimulated cells, NBMI decreased the slope of the uptake curve (results not shown).

If phosphorylation is the rate-limiting step in the uptake of nucleosides by quiescent cells, more of the intracellular pool of uridine should be phosphorylated in serum-stimulated cultures than in the control ones, after a short exposure to labelled nucleoside. The phosphorylated nucleosides would be trapped inside the cell. We tested this possibility by measuring the radioactivity associated with the cells after labelling the cultures with ^3H -uridine for 5 s, rapidly replacing the radioactive medium with non-radioactive medium containing 1 mM uridine and incubating for another 10 s to allow for efflux of free ^3H -uridine. We found that 67.4% of the radioactivity remained associated with the cells in serum-treated cultures while 45.9% remained so associated in the non-stimulated cultures, a difference consistent with the prediction. To substantiate directly this conclusion, acid-soluble pools were labelled with high-specific activity ^3H -uridine for 6 s and were then analysed chromatographically. That intracellular phosphorylation rather than transport is rate-limiting in nucleoside uptake by non-stimulated cultures (Fig. 3a) is demonstrated by the fact that NBMI added at a low concentration (0.5 nM, which inhibits total uptake only by 25%) selectively reduces the labelling of the free-nucleoside pool by about 50% but does not modify the amount of radioactivity measured in the uridine-phosphate pool (Fig. 3b). If transport were rate-limiting, the inhibition of labelling by NBMI should be equal in both pools (free and phosphorylated uridine). Addition of serum to quiescent cultures changes considerably the composition of the acid-soluble pools (Fig. 3c); serum increases the radioactivity recovered in the nucleoside phosphate pool and considerably reduces that corresponding to the pool of cellular free uridine (Fig. 3c). Clearly, serum stimulates the rate of conversion of ^3H -uridine to phosphorylated nucleosides. Similar results were obtained in four independent experiments. The percentages of radioactivity found in the phosphorylated pools were $48 \pm 5\%$ and $88 \pm 4\%$ in the control and serum-activated cultures respectively, which are consistent with the washout experiments described here.

Taken together, our experiments support the notion that the stimulation of uridine uptake by serum in density-inhibited 3T3 cells results from an increase in the rate of internal phosphorylation rather than that of transport. How external growth-promoting factors regulate this rapid internal process warrants further investigation.

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Activation of melanoma tyrosinase by a cyclic AMP-dependent protein kinase in a cell-free system

REGULATION of pigmentation is of considerable importance throughout the animal kingdom. Photoprotection, social behaviour, and a number of disorders like albinism, vitiligo, and melanoma are affected by the amount and distribution of melanin in the organism. Melanin is synthesised in melanosomes beginning with the oxidation of tyrosine to dihydroxy-phenylalanine (DOPA) by the enzyme tyrosinase. In Cloudman S91 melanoma cells, tyrosinase activity and melanisation are stimulated by elevated intracellular levels of cyclic AMP¹, apparently through the inactivation of an inhibitor of tyrosinase². We initiated a series of experiments to identify the molecules controlling tyrosinase activity. Kuo and Greengard's observations on the widespread occurrence of cyclic AMP-dependent protein kinases throughout the phyla suggested that protein phosphorylation reactions might be involved³. We describe here a cell-free system in which tyrosinase is activated following the addition of a cyclic AMP-dependent protein kinase isolated from melanoma cells. The kinetics of the reaction in the cell-free system are similar to those in intact cells. The activation of tyrosinase is completely dependent on the protein kinase and is enhanced by cyclic AMP, ATP, and magnesium. The activation involves the removal of an inhibitor of tyrosinase; in addition, a phosphoprotein phosphatase is present which is distinct from the inhibitor but which appears to antagonize the kinase-mediated reaction.

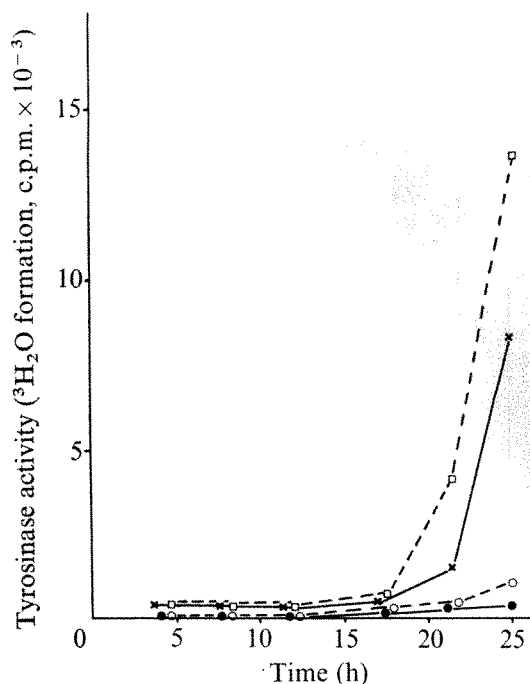
A cell line designated PS1-HGPRT-1 (ref. 4) was chosen for these studies because of its low basal tyrosinase activity which increases 5-50-fold about 15 h after the addition of melanocyte stimulating hormone (MSH) or dibutyl cyclic

Table 1 Lack of effect of kinase on tyrosinase activity in extracts of cells exposed to MSH before collection

Time of incubation (h)	Additions to reaction	Tyrosinase activity (^3HOH production c.p.m.)
0	None	9,670
	Kinase	9,242
24	None	10,800
	Kinase	7,930

Cells were cultured for 5 d with MSH (2×10^{-7} M) present in the medium. They were collected and an S30 fraction was prepared as described in the legend to Fig. 1. Each reaction tube contained S30 (0.14 mg ml^{-1}), cyclic AMP (10^{-6} M), ATP (2×10^{-4} M), and magnesium acetate (1×10^{-3} M) in Tris-HCl (50 mM, pH 6.2). Protein kinase, when added, was at a concentration of 0.16 mg ml^{-1} . Reactions were incubated at 30°C , and at the times indicated 20 μl -samples were withdrawn and assayed for tyrosinase activity as in Fig. 1.

Fig. 1 Activation of tyrosinase in a cell-free system. Cells were cultured in monolayer as described previously⁶. Log-phase cultures were fed fresh medium within 24 h of collection. They were collected with 5 mM EDTA in Ca-Mg free Tyrodes salt solution at room temperature, collected by centrifugation (2,500g for 5 min), and washed three times in NaCl (156 mM, room temperature) by successive centrifugations. The washed pellets of cells were resuspended in 10 volumes of ice cold Tris-HCl (25 mM, pH 7.4) containing 0.5% Triton X-100, and lysed by mixing gently with a Vortex Genie mixer every 2-3 min. over a 20-min period. The lysed cells were centrifuged (30,000g, 15 min, 4 °C), the pellets were discarded, and the supernatant solution (S30) was dialysed against 2,000 volumes of Tris-HCl (25 mM, pH 7.4) for 5 h at 4 °C. The dialysed S30 fraction was then stored at -90 °C in convenient aliquots where it remained stable for at least 6 months. The S30 fraction contained 0.8 mg ml⁻¹ protein as determined by the method of Lowry *et al.*⁷. Cyclic AMP-dependent protein kinase (also referred to as kinase) was partially purified from tumours originating from the same cell line inoculated into DBA 2J mice. Tumours were removed from mice, rinsed with NaCl (156 mM), and homogenised in 3 volumes ice-cold KPO₄ (5 mM, pH 6.8). All further steps were at 4 °C. The homogenate was centrifuged first at 3,000g for 5 min to remove large particulate matter, and then at 30,000g for 15 min. An equal volume of hydroxyapatite (0.5 gml⁻¹ in 5 mM NaPO₄, pH 6.2) was added to the 30,000g supernatant fraction, the mixture was stirred (5 h), and then centrifuged (30,000g, 15 min). More than 90% of the cyclic AMP-dependent protein kinase activity was associated with the hydroxyapatite while all tyrosinase activity remained in the supernatant fraction. Kinase was eluted by suspending the hydroxyapatite in three volumes KPO₄ (0.3M, pH 6.8, containing mercaptoethanol, 4 mM, and glycerol, 10%), stirring (15 h, 4 °C), and then centrifuging (30,000g, 15 min 4 °C). The supernatant fraction contained the kinase activity and was dialysed against 1,000 volumes buffer A (KPO₄, 10 mM, pH 6.8, mercaptoethanol, 2 mM, glycerol, 10%) overnight at 4 °C and then placed on a DEAE-cellulose column equilibrated with the same buffer. Kinase was eluted from the column with a salt gradient from buffer A alone to buffer A containing KPO₄ (0.3 M, pH 6.8). Two prominent peaks of kinase were eluted from the column, peak I containing more than 80% of the kinase activity and peak II containing the remainder. This kinase elution profile was essentially the same as that seen in other tissues by several groups (for example, see ref. 8). Fractions containing peak I kinase were pooled and made 60% with (NH₄)₂SO₄. The kinase precipitated in these conditions and was stored in aliquots at 4 °C where it remained stable for at least 8 months. Although this procedure resulted in a substantial purification of the kinase, the preparation contained 10-15 protein bands when analysed by SDS-acrylamide gel electrophoresis. The preparation was free of tyrosinase activity, tyrosinase inhibitors, and protein phosphatase. The cell system contained the following components: S30 fraction (0.16 mg ml⁻¹). Tris-HCl (50 mM, pH 6.2), gentomycin (0.05 mg ml⁻¹), and where noted kinase (0.14 mg ml⁻¹), cyclic AMP (10⁻⁶ M), ATP (2 × 10⁻⁴ M), and magnesium acetate (10⁻² M). At times indicated samples were withdrawn and assayed for tyrosinase activity. The tyrosinase assay was a modification of the method of Pomerantz⁹. Samples (20 µl) were mixed with 10 µl of a solution containing ³H-tyrosine (50 µCi ml⁻¹, 1.5 × 10⁻⁴ M), dihydroxyphenylalanine (dopa, 2.5 × 10⁻⁴ M) and NaPO₄ (5 × 10⁻² M, pH 6.8). The mixture was incubated at 37 °C for 20 min, and 1 ml of activated charcoal (100 mg ml⁻¹ in citric acid, 0.1 M) was added. This mixture was then mixed briefly with a Vortex Genie mixer, centrifuged (2,000g, 5 min) and the supernatant fraction containing ³HOH was poured over a Dowex 50 column (1 cm in a Pasteur pipette) equilibrated with 0.1 M citric acid. The column eluate was counted in a liquid scintillation counter. Tyrosinase activity was linear over the 20 min incubation at 37 °C and was enhanced about fivefold by the addition of dopa. Control (●), added cyclic AMP, ATP, Mg²⁺ (○), added kinase (×), added kinase and cyclic AMP, ATP, Mg²⁺ (□). These experiments were repeated more than 10 times with similar results each time.



AMP (Bt.cAMP) to the culture medium. A 30,000g supernatant (S30) fraction of these cells was prepared and incubated with or without the additions of cyclic AMP-dependent protein kinase, cyclic AMP, ATP, and magnesium. Tyrosinase activity increased dramatically after a lag period of several hours (Fig. 1). The increase was enhanced by cyclic AMP, ATP, and magnesium, all of which were necessary for maximum response. The optimal pH for the reaction was 6.2 and the optimal temperature was 30 °C. The kinetics of activation were similar to those observed in intact cells exposed to Bt.cAMP (Fig. 2). Cyclic AMP-dependent protein kinase activity was present in the S30 fraction, and if the concentration of the S30 was increased in the reaction mix, the activation reaction was no longer dependent on exogenously added kinase (data not shown). We first noticed the activation of tyrosinase when we inadvertently left a concentrated S30 fraction at room temperature and found the next day that the tyrosinase activity had increased by more than 100-fold.

When an S30 fraction was prepared from cells that had been exposed in culture to MSH for 5 d before collecting, the tyrosine activity was high in fresh extracts and no activation occurred following the addition of protein kinase (Table 1). In these experiments the addition of kinase actually caused a slight decrease in tyrosinase activity.

To gain additional evidence for the role of protein kinase in the activation of tyrosinase we investigated the effects

Table 2 Effects of kinase modulator on protein kinase and the activation of tyrosinase

Experiment	Additions	Protein kinase activity (ATP ³² incorporation, c.p.m.)	Tyrosinase activity (³ HOH production, c.p.m.)
a	Kinase only	1,100	—
b	Kinase + modulator	320	—
c	Cell-free system	—	7,400
d	Cell-free system + modulator added at zero time	—	750
e	Cell-free system + modulator added at 24 h	—	7,650

Protein kinase modulator was prepared from calf brain through step 2 of the procedure of Donnelly *et al.*⁹. Experiments a and b were measurements of cyclic AMP-dependent protein kinase activity. Each reaction mix contained NaPO₄ (50 mM, pH 6.8), kinase (0.14 mg ml⁻¹), ATP³² (10⁻⁴ M, 0.2 Ci mM⁻¹), cyclic AMP (10⁻⁶ M), magnesium acetate (10⁻² M), and histones as substrate (Sigma, calf thymus type 11A, 200 µg ml⁻¹). Mixes (100 µl) were incubated for 5 min at 30 °C and the reactions were stopped by the addition of ice cold trichloroacetic acid (TCA 10%). Precipitates were washed several times in TCA and counted in a liquid scintillation counter. Experiments c-e were measurements of tyrosinase activity after 24 h in the cell-free system described in Fig. 1. Each reaction contained S30 fraction, kinase, cyclic AMP, ATP, and Mg²⁺. Modulator was added at a concentration of 0.34 mg ml⁻¹.

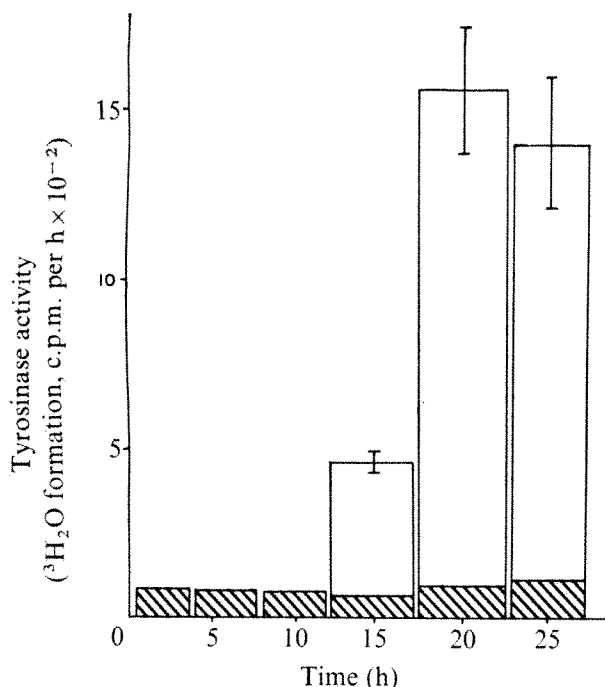


Fig. 2 Activation of tyrosinase in cultured melanoma cells by Bt₂cAMP. Cells (10⁶) were cultured in F10 medium (4 ml) containing 3,5-³H-tyrosine (New England Nuclear, 1 μ Ci ml⁻¹, final specific activity 88 Ci mol⁻¹) and at the times indicated 0.5-ml aliquots of medium were withdrawn and mixed with 1 ml charcoal as described in Fig. 1 to measure ³HOH. Counts represent the amount of ³HOH formed during each time period. This experiment was carried out in parallel to that presented in Fig. 1. Bt₂cAMP (10⁻³ M) (open bar); control (cross hatched bar).

of an inhibitor of cyclic AMP-dependent protein kinases (protein kinase modulator)⁹. Kinase modulator inhibited both the protein kinase and the activation of tyrosinase but had no effect on activated tyrosinase (Table 2).

The kinase-mediated activation of tyrosinase involved the removal of an inhibitor of tyrosinase. Freshly-thawed S30 fractions contained tyrosinase inhibitory activity which disappeared after the activation of tyrosinase. The inhibitor

Fig. 3 Decay of phosphatase activity at 30 °C. An S30 fraction was incubated at 30 °C and at the times indicated aliquots (20 μ l) were withdrawn, mixed with ³²P-labelled proteins described in the legend to Table 4, and phosphatase activity was measured.

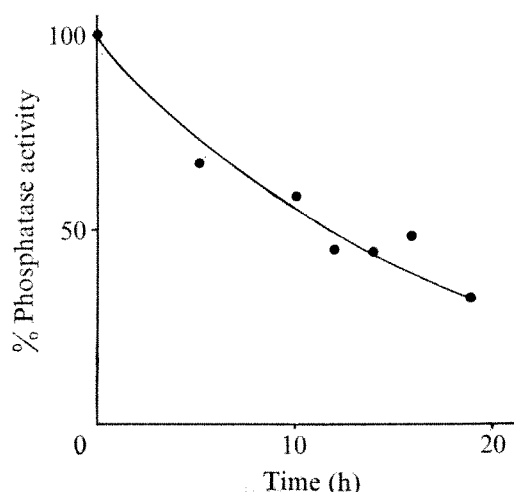


Table 3 Evidence for tyrosinase inhibitory activity in cell extracts*

Additions	Tyrosinase activity (c.p.m. ³ HOH production)
Buffer only (controls)	22,422
a, freshly-thawed S30 fraction	7,500
b, S30 fraction incubated 55 °C, 30 min	6,400
c, S30 fraction incubated 30 °C, 24 h	1,400

*A cell-free system containing S30 fraction, kinase, cyclic AMP, ATP, and Mg²⁺ was incubated 24 h at 30 °C as described in Fig. 1. At this time aliquots (10 μ l) were withdrawn and mixed with buffer only in controls or additional S30 fraction (10 μ l, 0.04 mg) which had been either a, freshly thawed; b, incubated at 55 °C for 30 min, or c, incubated at 30 °C for 24 h. ³H-tyrosine and dopa were added as in Fig. 1, the mixtures were incubated for 20 min at 37 °C and the amount of ³HOH produced measured.

activity was stable to heating at 55 °C for 30 min, or 30 °C for 24 h (Table 3).

Phosphoprotein phosphatase (referred to as phosphatase) activity was present in freshly-thawed S30 preparations (Table 4). The disappearance of acid precipitable ³²P counts was not due to proteolytic digestion of the ³²P-labelled phosphoproteins since ³H-leucine-labelled proteins mixed into the assay tubes were not degraded during the same time period. Phosphatase activity in the S30 fractions was destroyed by heating the fractions at 55 °C for 30 min. (Table 4) and had a half life of 11 h at 30 °C (Fig. 3). The kinase-mediated activation of tyrosinase took place after the phosphatase had decayed substantially (cf Fig. 1). This finding raised the possibility that the lag period in the activation of tyrosinase was due to an antagonism between kinase-mediated phosphorylations and phosphatase-mediated dephosphorylations. Evidence supporting this possibility comes from the finding that alkaline phosphatase inhibited the activation reaction (Table 5). Since the phosphatase activity was heat-sensitive and tyrosinase inhibitory activity was not (Table 3), it is clear that the two are distinct from one another.

Data from several experiments show that the control of melanisation in Cloudman S91 melanoma cells is complex (see ref. 1 for a review). MSH causes an increase in intracellular levels of cyclic AMP by binding to cell surface receptors in the G2 phase of the cell cycle and activating adenylate cyclase¹⁰. Results from the cell-free system described here indicate that elevated levels of cyclic AMP activate a protein kinase which in turn causes an activation

Table 4 Phosphoprotein phosphatase activity in cell extracts

Time of Incubation (min)	Acid precipitable ³² P-proteins (c.p.m.)	Acid precipitable ³ H-proteins (c.p.m.)
0	465	11,500
60	219	12,200
60, with heated S30	455	11,200

Substrate for the phosphoprotein phosphatase assay was prepared as follows: Freshly-thawed S30 was heated (55 °C, 30 min) to destroy endogenous phosphatase activity. A mixture was then prepared containing the heated S30 (0.16 mg ml⁻¹) protein kinase (0.14 mg ml⁻¹), ATP³² (10⁻⁵ M), magnesium acetate (10 mM), NaPO₄ (50 mM, pH 6.8) in a total volume of 5 ml. The mixture was incubated at 30 °C for 5 min, chilled on ice, and the ³²P-labelled proteins were separated from low molecular weight components on a Sephadex G25 column equilibrated with Tris-HCl (0.1 M, pH 7.4). Phosphatase activity was measured by adding the ³²P-labelled proteins to freshly thawed S30, incubating at 37 °C, and following the disappearance of acid precipitable ³²P counts on filter disks with time. To distinguish phosphatase activity from protease activity, ³H-leucine-labelled proteins isolated from cells cultured in ³H-leucine were mixed into the reaction tubes.

of tyrosinase. These findings suggest a simple model in which the kinase catalyses the phosphorylation of a tyrosinase inhibitor and thus inactivates that inhibitor. Proof for such a model can come only when the individual components of the system have been purified. Data were presented previously indicating that intact cells contain a tyrosinase inhibitor which is inactivated on exposure of the cells to MSH (ref. 2).

The following observations lead us to conclude that the events we have observed in the cell-free system are the same as those which occur in cultured cells. (1) Tyrosinase inhibitory activity is removed in both cases. (2) Extracts from cells pretreated with MSH have high tyrosinase activity and cannot be activated further in a cell-free system. And (3), the kinetics of activation are similar. It

Table 5 Effects of alkaline phosphatase on the activation of tyrosinase

	Tyrosinase activity (c.p.m. ^3HOH production)
Cell-free system	12,700
Cell-free system + alkaline phosphatase	3,500

A cell-free system containing S30 fraction, kinase, cyclic AMP, ATP, and Mg^{2+} was incubated for 24 h in the presence or absence of alkaline phosphatase (Boehringer Mannheim, calf intestine, 0.05 mg ml $^{-1}$).

should be noted that this report describes only the activation of tyrosinase, that is, the enzyme which catalyses the conversion of tyrosine to dopa. In none of the experiments did we see an increase in melanin formation, a result which we do not understand. In the melanin biosynthetic pathway, tyrosinase catalyses the oxidation of tyrosine to dopa and then of dopa to dopa quinone. Since the results reported here deal only with the first step in the reaction, it is possible that the second step is under separate controls and was not activated in our experiments. Using the cell-free system, we hope eventually to demonstrate increased melanisation and to identify the molecules controlling tyrosinase activity.

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Properties of an identified histamine-containing neurone

THE cellular role of neuronal histamine is still not understood, although a transmitter function is suspected¹. Advances have been restricted largely because of the lack of experimentally amenable, peripheral, histamine-containing systems in the vertebrates. As an alternative approach to the problem, we have sought an identifiable histamine-containing neurone in the central ganglia of a molluscan species. Previous studies on repeatedly identifiable neurones of this group have facilitated study of individual neurones known to contain, for instance, dopamine or 5-hydroxytryptamine, and the synaptic connections made by these neurones². Here we briefly describe the localisation of one particular histamine-containing neurone, its content of histamine, ability to take up histamine from an incubation medium, the type of granulated vesicles it contains and the influence of certain transmitter substances on its electrical activity.

Experiments were made on the pond snail, *Lymnaea stagnalis*. Neurones were initially screened using light microscope autoradiography after exposure to tritiated histamine, on the assumption that those neurones which contain histamine would also preferentially take up the amine from the extracellular fluid. The central ganglia were incubated at 18–20 °C in 100 μl of physiological solution³ containing 25 μM 2,5- ^3H -histamine dihydrochloride (1 Ci mmol $^{-1}$, Radiochemical Centre, Amersham) for 2 h. After washing in a continuous saline flow for 30 min, the tissues were fixed in 1% glutaraldehyde in 0.067 M phosphate buffer (pH 7.4), dehydrated through ethanol series and embedded in paraffin wax. Serial sections 7 μm thick, were coated with Kodak AR 10 stripping film, exposed for 10–14 d in the dark and developed in Kodak D 19. A specific population of neurones was found to be repeatedly labelled after this treatment. All of these neurones were small (<50 μm diameter) and not readily identifiable in intact preparations, with the exception of a single large neurone (Fig. 1) situated on the anterior dorsal surface of the visceral ganglion. The perikaryon of this neurone is 90–170 μm across its major axis and can be readily located in the majority of preparations by virtue of its size and position.

Histamine was assayed in extracts of individually dissected perikarya using a radioenzymatic method⁴. The

Fig. 1 Autoradiogram of a section of the visceral and left parietal ganglia of *Lymnaea* after incubation in physiological solution containing ^3H -histamine. Dense aggregations of silver grains are seen over the perikaryon of the giant visceral neurone (large arrow), and over portions of one of its main axon branches (small arrows) in the neighbouring left parietal ganglion. Scale bar represents 300 μm .



Table 1 Intracellular histamine concentration

Neurone	Intracellular histamine concentration $\pm$ s.e.m. (μ M)
Giant visceral neurone (<i>Lymnaea</i>)	275 ± 11.7 ($n = 4$)
Dopamine-containing neurone (<i>Planorbis</i>)	38.7 ± 3.8 ($n = 3$)
Non-aminergic neurone (<i>Lymnaea</i>)	None detectable ($n = 3$)

large neurone described above which took up histamine was found to contain a high level of histamine, whereas other identified neurones, a non-amine-containing neurone of *Lymnaea* (this neurone, located in a similar position to the VD1 of Winlow and Benjamin⁵, reacted negatively when the ganglia were examined by formaldehyde histo-fluorescence for primary amines) and the giant dopamine neurone of *Planorbis*⁶, contained much less histamine (Table 1). High specific histidine decarboxylase activity is associated with the part of the visceral ganglion containing the perikaryon of the histamine-rich neurone and recent experiments show that there is an enzyme within the perikaryon capable of synthesising histamine from histidine (J.D.T., unpublished).

For electron microscopy, individual neurones were dissected free from glutaraldehyde-fixed ganglia, post-fixed in 1% osmium tetroxide in 0.2 M veronal buffer (pH 7.4), stained in 1% uranyl acetate, dehydrated through acetone series and embedded in Epon. Examination of thin sections of the identified histamine-neurone by electron microscopy (Philips EM 301) revealed large aggregations of membrane bound dense-cored vesicles, of 80–200 nm diameter (Fig. 2), and extensive and highly organised rough endoplasmic reticulum in the cell cytoplasm, as well as numerous elongated mitochondria and lysosome-like bodies. The dense cores are larger than those of vesicles reported in the perikarya of molluscan dopamine-containing⁶ and 5-hydroxytryptamine-containing⁷ neurones. The vesicles also seem to occur in much larger aggregations than in these other amine-containing neurones.

The histamine neurone was readily located in the isolated ganglionic preparation for intracellular electrical

recordings using conventional techniques. Small excitatory postsynaptic potentials and inhibitory postsynaptic potentials were recorded from the neurone in different preparations, without applying any stimulation. Occasionally the excitatory input was sufficient to cause the cell to fire action potentials. The effects of some transmitter substances were tested on the activity of the neurone. These compounds were applied iontophoretically from fine-tipped micropipettes placed close to the perikaryon, using current pulses of 50–300 nA for durations of 0.5–2.5 s. Acetylcholine caused a biphasic depolarising–hyperpolarising response. The first phase of the response was comparatively fast and blocked with 10^{-4} M hexamethonium bromide. The second phase was not blocked with this concentration of hexamethonium; it was very rapidly desensitised and chloride dependent. 5-Hydroxytryptamine also produced a rapidly desensitising, chloride-dependent hyperpolarising response. In contrast, iontophoretically applied histamine, dopamine, glutamate and γ -aminobutyric acid were without any obvious effects. But addition of histamine acid phosphate directly to the perfusion fluid resulted in a gradual depolarisation of the neurone, due to an increase in membrane conductance, and spike firing.

The giant visceral neurone has numerous axon branches with ramifications in other ganglia. Some branches leave the central ganglia for the periphery (J.D.T., S. Logan and G.A.C., unpublished data). As yet, however, the functional role of the neurone is not known.

In summary, there is a giant histamine-containing neurone in the visceral ganglion of *Lymnaea stagnalis*. The histamine concentration in this neurone (275 μ M) is comparable with that recently reported for the C2 neurones (300–400 μ M) located in the cerebral ganglia of *Aplysia californica*⁸. The *Lymnaea* histamine-neurone contains large aggregations of granulated vesicles of distinctive appearance and it is capable of taking up histamine from the surrounding medium. The neurone can be readily located for electrophysiological experiments and would seem to provide a suitable model system for analysing the cellular role of neuronal histamine.

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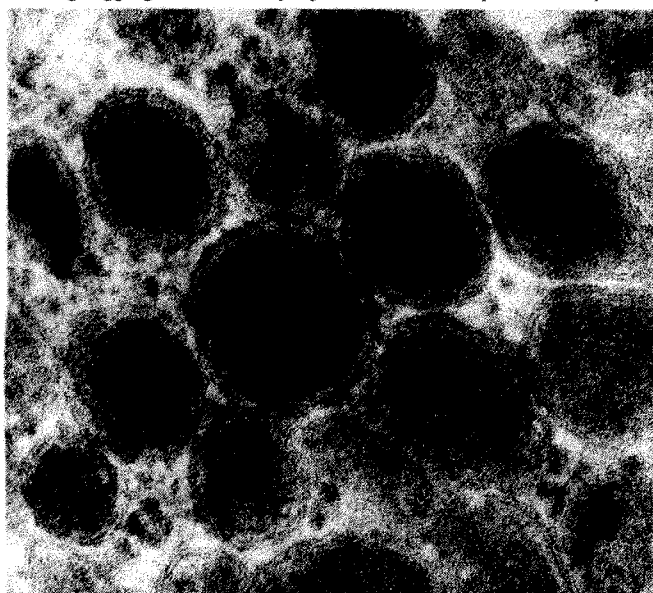
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Fig. 2 High magnification electron micrograph of the vesicular inclusions in the perikaryon of an isolated giant *Lymnaea* visceral neurone. The vesicles (80–200 nm diameter) have limiting membranes surrounding large electron-dense cores and are found in large aggregations in the cytoplasm. Scale bar represents 0.1 μ m.



5-Hydroxytryptamine receptors on visceral primary afferent neurones in the nodose ganglion of the rabbit

5-HYDROXYTRYPTAMINE (5-HT) is known to be a powerful activator of the sensory nerve endings¹ and a potent stimulant of sympathetic ganglia^{2–4}. Very little is known, however, about the nature of 5-HT receptors and the ionic basis of the 5-HT-induced membrane potential changes^{5,6}.

Here I report the existence of 5-HT receptors on the soma of afferent neurones of the nodose ganglion of the vagus and the ionic requirements of the response elicited by activation of these receptors.

The nodose ganglion was excised from the rabbits anaesthetised with pentobarbitone (30 mg per kg body weight intravenously) and intracellular recordings were made from the somata of neurones by methods essentially similar to that of Jaffe and Sampson⁷. 5-HT was applied either by superfusion as an addition to Krebs solution or by iontophoresis. Electrodes used for iontophoresis were filled with 0.1 M 5-HT adjusted⁸ to pH 3.8–4.0 and had tip resistances of 100–200 M Ω .

A majority of neurones (76 out of 88) were depolarised by 5-HT. Figure 1a shows a typical response to superfusion with a solution containing 5×10^{-6} M 5-HT. In most instances, the onset of the depolarisation was rapid and concentration dependent. The minimal effective concentration to produce the depolarisation was 10^{-7} M, and the maximal response was obtained at concentrations greater than 10^{-4} M. Repetitive firing frequently occurred during the rising phase of the depolarisation. The depolarisation was followed by an almost equally rapid repolarisation, then a hyperpolarisation (after-hyperpolarisation) of approximately 5 mV. The after-hyperpolarisation was in turn followed by a long-lasting depolarisation of a few millivolts.

Iontophoretic application of 5-HT elicited a response similar to that induced by superfusion. As seen in Fig. 1b, a 10^{-8} -A pulse (80 ms) sufficed to produce a depolarising response and a typical biphasic response was induced with a 10^{-7} -A pulse.

All the components of a 5-HT response were associated with a reduction of membrane resistance as by the decrease in the amplitude of the electrotonic potentials superimposed on the response (Fig. 1a).

In an attempt to determine the ionic species involved, the 5-HT responses were studied in various ionic media and at different membrane potentials. Replacement of external sodium by choline or Tris (hydroxymethyl)aminomethane abolished not only the rapid and residual depolarisations but also the after-hyperpolarisation. Removal of external potassium caused a slight decrease of the rapid depolarisation, a marked enhancement of the after-hyperpolarisation and no significant alteration in the residual depolarisation. Replacement of external chloride by acetate reduced the resting potential by approximately 15 mV and nearly halved the amplitude of 5-HT response. This reduction in amplitude was, however, eliminated when the membrane potential was restored by passing hyperpolarising current through the microelectrode.

When the membrane was depolarised stepwise, the rapid depolarising response was reduced accordingly and finally reversed at a certain level (-0.1 ± 2.0 mV s.e., $n=8$). This reversal level was at least 30 mV below the sodium equilibrium potential determined from the peak of the action potential. The after-hyperpolarisation could be reversed at -87 ± 2.5 mV ($n=8$) at which voltage the after-hyperpolarisation of the action potential also inverted. The reversal levels of the rapid depolarisation and the after-hyperpolarisation were unaltered by the replacement of external chloride with acetate.

These findings suggest that during the rapid initial depolarising response to 5-HT, there is a simultaneous increase in sodium and potassium conductance and during the after-hyperpolarisation only potassium conductance is increased. Since, however, both the initial depolarising and hyperpolarising responses are abolished in sodium-free media, the possibility arises that the after-hyperpolarisation is triggered by the entry of sodium ions. The fact that the residual depolarisation was abolished by deprivation of sodium but unaltered by withdrawal of potassium indicates

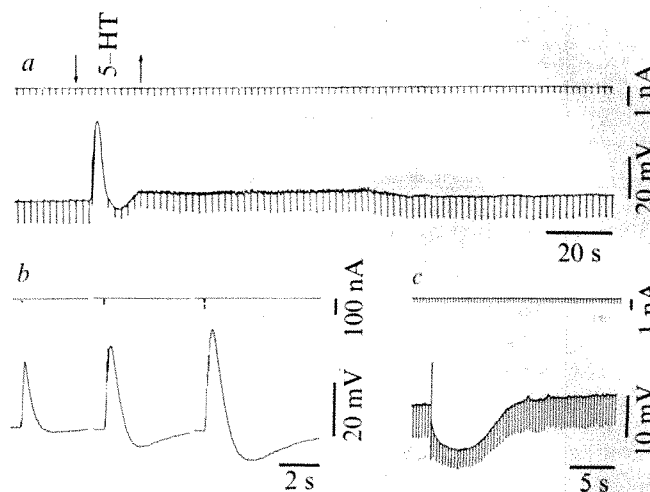


Fig. 1 5-HT-induced responses in afferent neurones of the rabbit nodose ganglion. Intracellular records from three different neurones show; *a*, depolarising response to 5×10^{-6} M 5-HT applied by superfusion for 20 s (between two arrows). Electrotonic potentials were elicited by square pulses (0.3 nA, 100 ms) every 2 s. *b*, Depolarising responses to 5-HT iontophoresed by current pulses (80 ms) of 20 nA (left), 35 nA (middle) and 85 nA (right). *c*, Hyperpolarising response to 5-HT iontophoresed by a current pulse of 80 nA and 100 ms. Electrotonic potentials were elicited by square pulses (0.25 nA, 50 ms) every 0.3 s. In each record the upper trace shows electrotonic (*a* and *c*) or iontophoretic (*b*) current pulses and the lower trace the membrane potential changes. Resting potentials of the three neurones were -58 mV (*a*), -62 mV (*b*) and -55 mV (*c*). Action potentials that appeared on the rising phase of 5-HT-induced depolarisation were attenuated by the ink-writing recorder.

that the residual component is associated with a small but sustained sodium permeability.

In a small number of neurones (5 of 88), 5-HT evoked a simple hyperpolarising response with no preceding or succeeding depolarisation as shown in Fig. 1c. The response varied in amplitude from 3.8 to 11.2 mV and in duration from 5.4 to 10.4 s and was consistently accompanied by a reduction of membrane resistance (Fig. 1c). Removal of potassium enhanced the hyperpolarisation, and displacement of membrane potential beyond -90 mV reversed its polarity. These characteristics reflect that an increased potassium conductance is the major, if not the exclusive, cause of this hyperpolarising response.

A similar group of neurones (7 of 88) did not show any response to 5-HT applied by iontophoresis and by superfusion.

Thus the majority of vagal afferent neurones are endowed with 5-HT receptors which are most commonly depolarising and only rarely hyperpolarising. The functional role of these receptors is obscure at the present. Presumably they could be a remnant of an embryonic stage in which 5-HT receptors migrate from the cell soma to the axon terminals. A number of authors have suggested that the depolarising response of the same population of neurones to GABA is similarly mediated by residual receptors (ref. 9). Much in the same way the 5-HT receptors would offer an opportunity to investigate their physiological and pharmacological characteristics in conditions which cannot as yet be achieved at the axon terminal.

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A regenerating neurone in the leech can form an electrical synapse on its severed axon segment

WHEN the nervous system develops, its constituent neurones establish connections with certain selected elements while rejecting others in a fashion that gives the completed system the precision it requires to function. Such specificity is also manifested during regeneration in the central nervous system (CNS)¹⁻³. For example, regeneration and recognition have been demonstrated with electrophysiological techniques in the leech CNS^{4,5}, where individual sensory neurones can select their normal postsynaptic targets from among apparently hundreds of alternatives, re-establishing chemical synaptic connections. The leech CNS has also provided an example of neurones re-establishing electrical continuity after surgical disruption of a connecting axon⁶. Such electrically connected 'S-cell' interneurons, one of which is situated in each of the 21 segmental ganglia of the leech, are linked at the ends of their axons by electrical synapses across which large molecules cannot pass⁷. The synapse between two S interneurons occurs in the axon bundles (connectives) that join ganglia. The discovery of this junction and the use of the enzyme horseradish peroxidase (HRP) as an intracellular marker have permitted a detailed morphological and physiological analysis of the steps by which a regenerating axon re-establishes synaptic contact with its proper target. We report here that one such step can be the formation of an electrical synapse between the regenerating neurone and its own severed axon segment, thereby restoring the neurone's functional integrity.

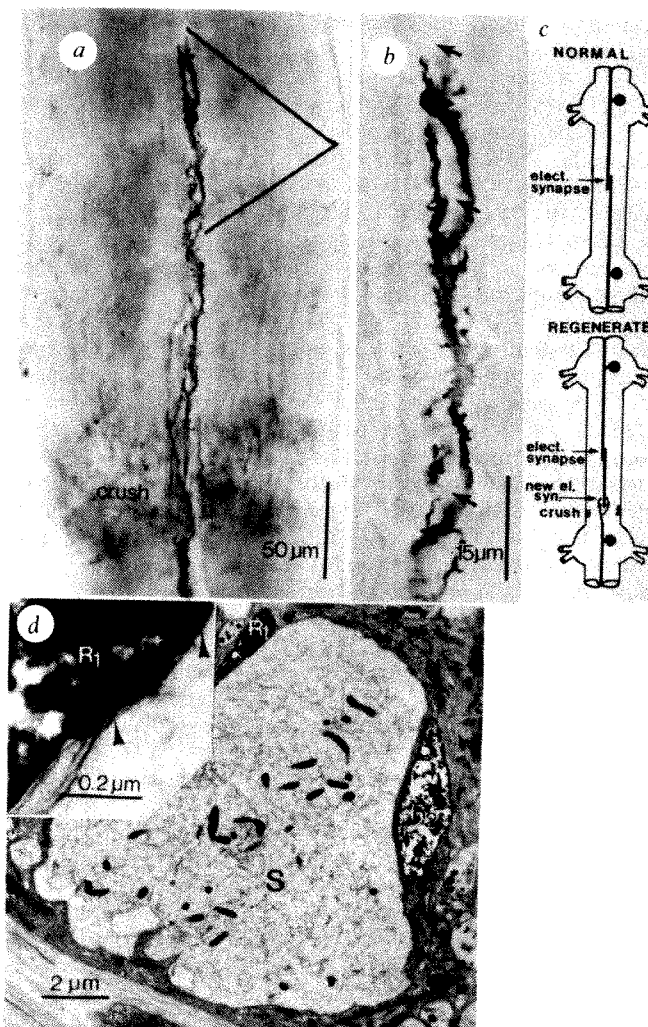
Except at the extreme ends of the ventral nerve cord, the axons of S cells meet at about the midpoint along the connectives between the ganglia (Fig. 1c). This single region of contact between neighbouring S cells is the site of the electrical synapse, across which nerve impulses can pass in either direction as they traverse the entire length of the intact nerve cord. In the present experiments the cord was crushed close to one ganglion (either 8, 9, 10 or 11) so that the electrical synapse between S cells was not directly affected by the lesion. This procedure severs all axons including those in the bundle of nearly a hundred in which the S-cell axon travels. Animals were allowed to recover in spring water at 16 °C, the usual holding temperature. Distal to the crush the operation leaves an isolated axon segment about 1 or 2 mm long that remains viable and linked through an electrical synapse to the adjacent S cell, termed here the 'target' cell of the regenerating process.

The extent of regeneration was determined by removing a short chain of ganglia including the lesion from the animal, and then recording from the chain in the leech Ringer's solution^{8,9}. Electrical coupling between cells was detected with intracellular microelectrodes inserted into S-cell somata and with suction electrodes applied to the connectives. Intracellular microelectrodes were filled either with 4 M potassium acetate or with a solution of 2% horseradish peroxidase in 0.2 M potassium chloride. To mark the entire arborisation of an S cell, peroxidase solution was injected under pressure into the S-cell soma through the recording microelectrode, the chain of ganglia incubated for 6-12 h in a modified L-15 culture medium¹⁰⁻¹¹, and the histochemical reaction and subsequent treatment for light or electron microscopic observation performed as previously

described¹²⁻¹³. The diaminobenzidine reaction product is not seen in uninjected cells that are electrically coupled to the injected neurone, but owing to the activity of endogenous peroxidases some reaction product can appear in peroxisomes in all cell somata, especially in culture medium.

In six experiments the restoration of electrical coupling and subsequent conduction of impulses between S cells occurred in less than 4 weeks. In these preparations there was no anatomical contact between neighbouring S cells, as determined with the HRP marking technique. Rather, when HRP was injected into both the regenerating injured S cell and its target S cell (which showed no sign of growth) the reaction product revealed a gap as large as a millimeter between the stained axons (Fig. 1a). This distance decreased during the month following the lesion. In these instances

Fig. 1 The regenerating axonal processes of the S cell surround the neurone's severed distal segment. *a*, The axon proximal to the crush (below) branches as it crosses the crush in the centre of the connective and remains branched as it forms a basket of processes along the site of the distal segment. The marked cell ends abruptly several hundred micrometres before reaching the next S cell (not shown) whose extent had also been determined with peroxidase injections. The growing tip is shown in *b*. In this plane of focus the marked processes are seen to surround portions (indicated at three locations by arrows) of the distal segment (18 d). *c*, Schematic diagrams from peroxidase-injected preparations indicating the normal and experimentally induced connection (anterior at top). *d*, Electron micrograph of connective cross section showing two stained processes of the regenerating neurone (R_1 and R_2) near the distal segment (S), identified by its large size, dorsal position in Faivre's nerve and light cytoplasm. Inset: where the regenerating axon (R_1) apposes the segment are points at which the membranes are separated by no more than 4-5 nm (arrowheads), the presumed gap junctions (34 d).



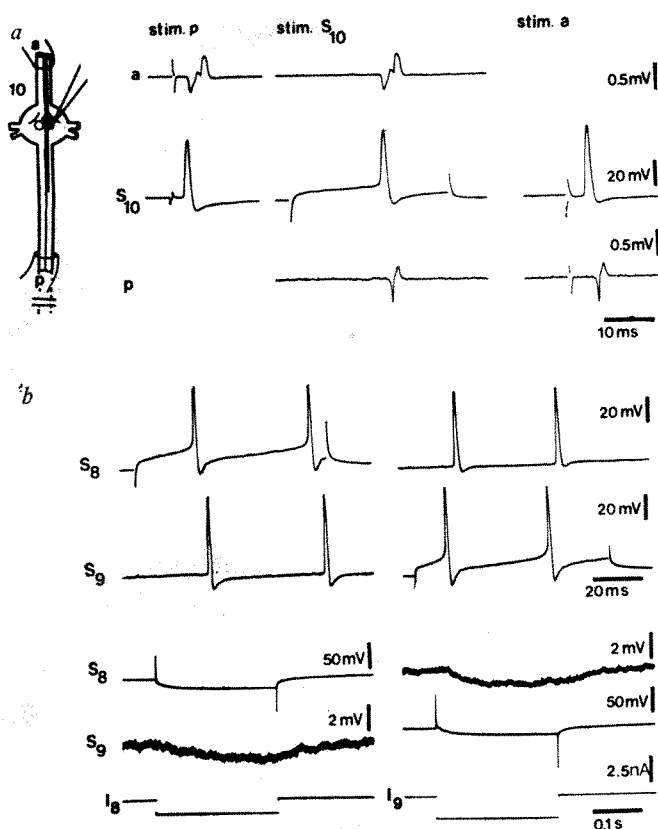


Fig. 2 *a*, The distal axon segment (here of S cell in ganglion 11) remains functionally connected to the adjacent target S cell (in ganglion 10) after crushing close to ganglion 11 (19 d previously). Suction electrodes record anteriorly (*a*) and posteriorly (*p*) to ganglion 10, in which the target S cell (S_{10}) has been penetrated with a recording microelectrode. Prior recording and stimulation posterior to the crush (=) demonstrated that the S-cell connection from ganglion 11 had not regenerated. The recordings here illustrate respectively that (*stim. p*) threshold stimulation at *p* excites S_{10} whose impulse is also recorded at *a*; (*stim. S₁₀*) direct activation of S_{10} with a depolarising pulse of current through the recording microelectrode activates not only its own axon recorded at (*a*), but also the axon of the distal stump recorded at *p*; and (*stim. a*) anterior connective stimulation at threshold directly activates S_{10} and subsequently the distal stump at *p*. In the diagram on the left, the thickened line in the connectives represents the peroxidase-stained axon of S_{10} , which terminated more than one millimetre from point *p*. At this distance the suction electrode (*p*) would not have directly activated or recorded from S_{10} . *b*, Coupling of S-cells through the distal segment. The connective between ganglia 8 and 9 was crushed 20 d previously. S cells (S_8 and S_9) that, on subsequent marking with peroxidase, were found not to have regenerated a direct contact nevertheless show normal functioning. Upper: impulses generated in S_8 with the recording microelectrode are propagated to S_9 and vice versa. Lower: hyperpolarising currents injected into S_8 or S_9 spread into the adjacent S cell, indicating good coupling through the uninjected process.

of coupled neighbours not in direct contact, the regenerating neurone formed a basket of processes around a 5–8- μ m cylinder that in the electron microscope is identified as its own distal segment (Fig. 1*b, c* and *d*). Glial processes are seen between most of the regenerating and old axons, but at the sites of contact between the regenerating process and the distal segment are occasional points of close apposition. Tilting of the specimen in the electron microscope reveals that the membranes are separated by no more than 4–5 nm. These areas of contact, though small, resemble gap junctions between neighbouring S cells in the normal connective⁷ and those found in other invertebrates¹⁴. Electron microscopic examination of cross sections through the connective in the region of the discontinuity between injected S cells confirms that S-cell regeneration is not complete and that the distal axon segment is linking the S cells.

During the month after the lesion is made the distal axonal segment can retain its integrity and remain functionally connected to the target S cell through the old electrical junction. The S-cell axon normally has a low threshold and generates the largest impulses recorded from the connective. These physiological properties and nearly normal ultrastructure are retained by the distal axon segment during the first month. Figure 2*a* shows that before the two S cells have become functionally reconnected the isolated distal segment can still excite and be excited by the adjacent target S cell.

In two or three weeks the coupling between adjacent S cells through the isolated axonal link develops a nearly normal⁶ strength with impulses conducted in both directions (Fig. 2*b*). This coincides with an increasingly extensive association between the regenerating neurone and its distal segment, which itself remains connected to the target S cell.

When coupled neurones are examined at progressively later times, the distance shortens between the axon tips of the injected adjacent S cells. For example, the cell in Fig. 1*d* had grown 700 μ m to within 150 μ m of the target S cell. After about 1 month no separation between coupled cells is seen. This suggests that neurones already functionally connected through the distal segment can eventually re-establish direct contact at the normal site of synapse through continued regeneration of the lesioned neurone. Growth beyond the normal synaptic contact has not been observed even after six months.

Regenerating S cells establish strong electrical synapses specifically with S cells. In several experiments electrical coupling was not detected between the regenerating S cell in one ganglion and cells near the S cell in adjacent ganglia. Moreover, an impulse in the regenerating S cell will evoke only an S-cell impulse recorded in the connectives. Certainly the close anatomical association between the regenerating S cell and its own distal segment suggests a specific and exclusive recognition of cell type.

The electrical synapse formed by the regenerating neurone on its own distal segment is functionally indistinguishable from a direct fusion with the segment, except that large molecules such as HRP (molecular weight, 40,000) are unable to cross the junction. This raises the possibility that for other systems in which fusion has been invoked to explain rapid and complete regeneration (see refs 15–17 and compare ref. 18), an electrical synapse has also formed.

Knowledge of the exact point of synapse between neighbouring, electrically coupled S neurones in the leech has provided a tool for following a regenerating neurone as it re-establishes a fully functional and structurally intact electrical synapse. In some cases transmission was rapidly restored when the regenerating neurone formed first an electrical synapse with its own severed but still functioning distal axon segment. The segment seems to be an imperfect target for the regenerating neurone, however, for the neurone continues growing along the axon segment to the normal site of synapse, where growth ceases.

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Cross-tolerance between morphine and cholinergic blocking agents microinjected into anterior amygdala

WE have previously demonstrated tolerance to repeated applications of opiates microinjected directly into the amygdaloid region of the brain¹. Since morphine has been shown to inhibit the release of acetylcholine at some brain sites^{2,3}, as well as in the isolated guinea pig ileum^{4,5}, we compared the effect of morphine on amygdala bioelectric patterns with the effects of hemicholinium and scopolamine—two drugs that interfere with cholinergic synaptic activity in different ways. Tolerance that is produced with repeated administration of morphine is associated with cross-tolerance to hemicholinium and scopolamine.

Eighteen rats were anaesthetised with sodium pentobarbital 50 mg kg⁻¹, and bilateral cannula-recording electrode assemblies were implanted in the anterior amygdala. The permanently implanted cannula serves as a guide for

Fig. 1 Development of morphine tolerance to repeated daily microinjections (0.9 µl) of morphine sulphate 10 µg µl⁻¹ (M10) into the anterior amygdala of rat 460. Some recovery of the response was observed when the morphine concentration was increased three times (M30). ▲, M10 dose 1; ★, M10 dose 2; ×, M10 dose 4; ■, M10 dose 9; ●, M30 dose 10; —, no drug.

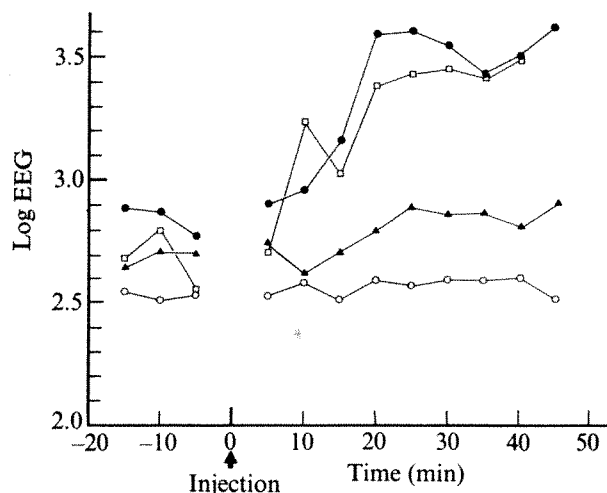
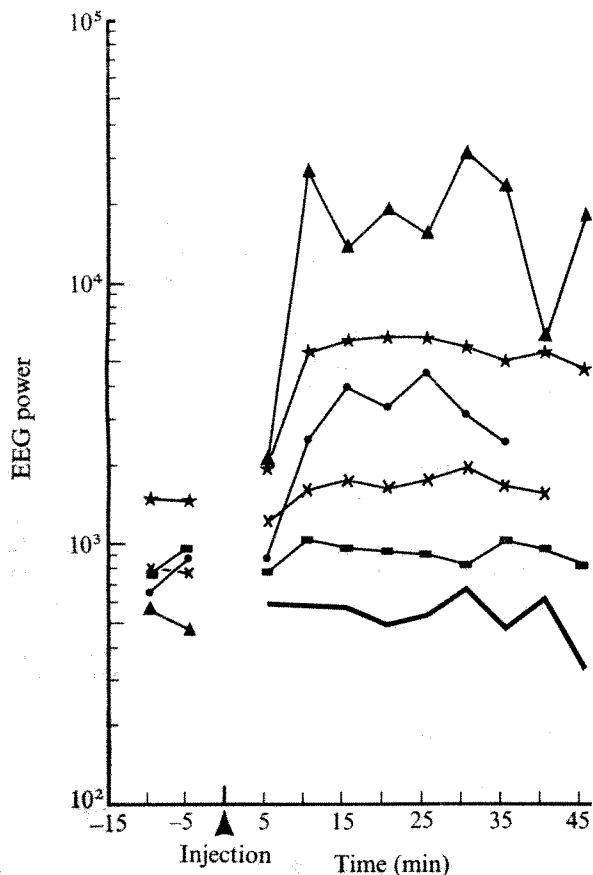


Fig. 2 Cross-tolerance between morphine 10 µg µl⁻¹ and hemicholinium 5 µg µl⁻¹ microinjected into left anterior amygdala of rat 488. Tolerance to hemicholinium was observed after ten consecutive microinjections. The hemicholinium-tolerant amygdala was also tolerant to morphine. ●, Morphine dose 1; □, hemicholinium dose 1; ▲, hemicholinium dose 10; ○, morphine dose 2 after hemicholinium dose 10.

smaller fluid delivery cannulae and as one pole of the bipolar recording assembly that monitors changes in bioelectrical activity at the injection site. All injections were delivered at a rate of 0.03 µl s⁻¹ for 30 s. In a previous report¹ we showed that analogue recordings from the injection site show spike and wave patterns in response to morphine sulphate but not to injections of saline and dextrophan, the ineffective (+)-isomer of levorphanol. Tolerance developed after repeated daily injections of morphine or levorphanol, whereas no change in morphine sensitivity was observed after repeated injections of saline. The morphine-responsive area was localised to a region ventrolateral to the anterior limb of the anterior commissure. This response to morphine was not observed at injection sites above or below this region or at medial thalamic loci. Haloperidol 6 µg µl⁻¹ had no effect when injected into morphine-responsive sites.

In the present experiment, the analogue recordings were subjected to digital conversion, and the root-mean-square value of EEG amplitude was computed for 5-s epochs taken at successive 5-min intervals before and after the unilateral injections.

To avoid confounding the drug response with high amplitude activity associated with sleep or drowsiness, the rats were maintained on a 22-h water-deprivation schedule. They were trained to consume their normal water intake (approximately 25 ml) by drinking from a water bottle spout that moved into an aperture at one end of the recording chamber on an aperiodic schedule, providing the rat with access to water for 3 s at a time. All the rats learned to consume their daily fluid intake in this manner before the recording sessions were started. Typically, each rat would sit quietly facing the aperture until it was time to drink. This procedure was very effective in providing a means of maintaining a steady EEG baseline throughout the recording session (for example, see Fig. 1).

Microinjections (0.9 µl) of morphine 10 µg µl⁻¹ caused the amplitude of bioelectric activity at the injection site to change from a mean of 567 µV² (s.d. 172) to a mean of 3,982 µV² (s.d. 1,925). This change was statistically significant ($P < 0.01$).

The progressive loss of sensitivity of the amygdala to repeated daily doses of morphine is shown in Fig. 1. Within 10 min after the first injection, the amplitude of the signal at this injection site increased to more than 10

times baseline values and persisted throughout the 45-min recording session. Tolerance, manifested by diminished responsiveness, developed in increments with each successive daily morphine treatment. In the experiment presented in Fig. 1, no observable response to morphine was seen on day 9. Increasing the concentration of morphine to $30 \mu\text{g } \mu\text{l}^{-1}$ on d 10, however, resulted in the reappearance of the morphine response from the tolerant amygdala (Fig. 1).

Cross-tolerance tests between morphine and cholinergic blocking drugs were carried out in 18 animals. In ten animals, tolerance to morphine was established first; in five, tolerance to scopolamine was established and morphine sensitivity was evaluated. Three were first made tolerant to hemicholinium and then tested with morphine. In every case, the first dose given was morphine, followed by a test dose of either scopolamine or hemicholinium.

In tests of cross-tolerance, in which morphine tolerance was first established, cross-tolerance to hemicholinium and scopolamine was found in eight out of ten cases. In two cases the response to scopolamine was reduced but still evident in morphine-tolerant subjects. Tolerance to morphine was observed in every case in which tolerance to scopolamine ($n=5$) and hemicholinium ($n=3$) had been previously established.

An experiment where hemicholinium tolerance was first established is shown in Fig. 2. First, the left amygdala of rat 488 was tested for sensitivity to morphine $10 \mu\text{g } \mu\text{l}^{-1}$ and then to hemicholinium $5 \mu\text{g } \mu\text{l}^{-1}$. A robust response was observed shortly after microinjection of each drug on

successive days. This injection site became tolerant to hemicholinium by the tenth dose of this drug. When the hemicholinium-tolerant injection site was retested for sensitivity to morphine, cross-tolerance to morphine was evident (Fig. 2).

Tolerance to scopolamine is demonstrated in Fig. 3a. The response to morphine before and after the development of scopolamine tolerance is shown in Fig. 3b. When morphine sensitivity was tested at this scopolamine-tolerant injection site, cross-tolerance to morphine was observed (dose 2, Fig. 3b).

Tolerance to morphine and cholinergic blocking drugs is not the result of an irreversible alteration of nerve cells in the anterior amygdala. After tolerance has been acquired, if the injection site is left alone for about 3 weeks, some recovery in the sensitivity to these drugs can be observed. This phenomenon is demonstrated in Fig. 3, in which tolerance to scopolamine and cross-tolerance to morphine was established in the right amygdala of rat 502.

When sensitivity was tested after a 25-d layoff, baseline recordings before injection remained stable, but tolerance to both scopolamine and morphine was no longer evident; the drug response was observed again ($n=3$).

In view of negative findings⁶⁻⁸, it is unlikely that cross-tolerance between opiates and cholinergic blocking drugs could have resulted from a change in the number or affinities of opiate receptor-binding sites.

It is possible that the chronic blockade of synaptic activity by morphine and the cholinergic blocking drugs used in this experiment caused a proliferation of postsynaptic neurotransmitter receptor sites⁹, producing both supersensitivity and cross-tolerance at the injection site. The relationship between disuse supersensitivity and tolerance has been discussed in detail by Jaffe and Sharpless¹⁰, and has been amply demonstrated^{11,12}.

Although cross-tolerance between systemic injections of morphine and muscarinic blocking drugs has been shown previously¹³, this is the first demonstration of symmetrical cross-tolerance between morphine and cholinergic blocking drugs microinjected directly into a discrete brain region.

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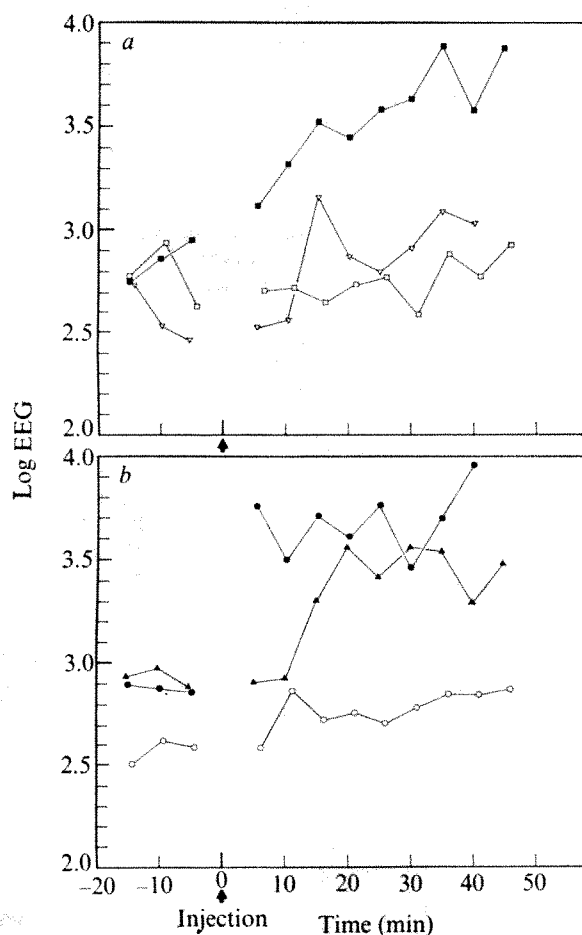


Fig. 3 Tolerance to scopolamine ($18 \mu\text{g}$ in $0.9 \mu\text{l}$) after 20 daily microinjections into the right anterior amygdaloid region of rat 500 (a). ■, Dose 1; □, dose 20; ▽, after 25-d layoff. Cross-tolerance to morphine ($9 \mu\text{g}$ in $0.9 \mu\text{l}$) at this injection site is also shown (b). ●, Dose 1; ○, dose 2; after 26-d layoff. Recovery of sensitivity to both drugs was observed after a 25-d abstinence period.

Electron spin resonance study of membrane protein alterations in erythrocytes in Huntington's disease

HUNTINGTON'S disease (HD), a degenerative disease of the central nervous system inherited as an autosomal dominant trait, is characterised clinically by progressive choreiform movements and dementia¹. Pathological changes include atrophy, severe cell loss and gliosis in the caudate nucleus and putamen and to a lesser extent the globus pallidus and cerebral cortex². The aetiology and pathogenesis of this disease are not understood. Although HD is considered a

defect of the basal ganglia and cerebral cortex, we report here evidence obtained by electron spin resonance (ESR) techniques (methods reviewed in ref. 3) which suggests HD may have more widespread membrane involvement.

Understanding of the basic biochemical defect in HD has not progressed greatly in recent years. Early biochemical studies performed in HD have been reviewed⁴. More recent studies have shown that the inhibitory synaptic transmitter γ -aminobutyric acid (GABA) and a related dipeptide, homocarnosine, were reduced in autopsied HD basal ganglia⁵. Reduced activity of glutamic acid decarboxylase, the enzyme involved in the synthesis of GABA, was found in post-mortem basal ganglia of HD patients⁶. Choline-acetyltransferase, the biosynthetic enzyme for acetylcholine was also reported to be diminished⁶. Other studies have shown elevated levels of ribosomal proteins⁷ and lipofuscin pigment⁸ in HD neurons.

One of the major obstacles to biochemical studies of HD is that the primary alterations which give rise to its characteristic symptoms are found in the basal ganglia and cerebral cortex. Consequently, the availability of significant amounts of fresh, repetitively obtainable, useful specimens for study has been limited. Other uncontrollable factors such as delay in obtaining autopsy tissue, use of various drugs by patients, and super-imposed ageing changes or other pathological alterations have limited the value of such tissue and the enthusiasm of research in this area.

Similar considerations of sample purity and secondary biochemical changes on denervation of muscle led to the study of erythrocyte membranes in several inherited neuromuscular diseases. Our previous spin labelling studies⁹⁻¹⁵ have suggested that myotonic and Duchenne muscular dystrophy and congenital myotonia are diffuse membrane diseases even though muscle is the primary tissue in which symptoms are expressed. An increased membrane fluidity associated with myotonic states⁹⁻¹² and alterations in the membrane protein conformation and/or organisation in dystrophic states¹³⁻¹⁵ were suggested by these spin labelling studies.

We have performed similar ESR experiments with erythrocyte membranes from normal and HD patients. The principal spin label used was 2,2,6,6-tetramethylpiperidin-1-oxyl-4-maleimide (MAL-6) (Syva Associates). MAL-6 has been shown to covalently bind to sulphydryl (SH) groups although a small amount of amino group binding may also occur¹⁶. Fresh, heparinised blood was obtained from 10 patients with well-documented HD from 10 different families. Four of the patients were in the very early stages of the disease, three were in the mid-stages, and three were in the bed-ridden, late stages. Seven of the 10 patients were ambulatory and active. Five of the HD patients were on a regular at-home diet; the other five were on a regular hospital diet. Two of the HD patients were on no medication, two were on diazepam, three were on a butyrophenone and a phenothiazine, and three were on an assortment of medication. Individuals without clinical manifestations or family history of inherited neurological diseases served as controls. Additional ESR studies were undertaken on erythrocyte membranes from patients with non-neurological disorders who were on a butyrophenone and a phenothiazine or diazepam. Intact erythrocytes and erythrocyte membranes (ghosts) were obtained and the spin labelling performed as previously described¹³ except a 1:25 weight ratio of spin label to membrane protein was used. Magnetic resonance experiments were performed on a Magnion-Ventron MVR-9X ESR spectrometer using a quartz aqueous sample cell.

A typical spectrum of erythrocyte ghost membranes labelled with MAL-6 is shown in Fig. 1. Spectra similar to that of Fig. 1 have been previously described as reflective of at least two classes of membrane protein SH groups: one strongly immobilised and the other weakly im-

mobilised¹³⁻¹⁹. The ratio of the spectral amplitude of MAL-6 attached to weakly immobilised SH groups (*W*) to that of MAL-6 attached to strongly immobilised SH groups (*S*) is a convenient monitor of protein organisational and/or conformational changes in membranes¹³⁻¹⁹.

The mean *W/S* ratio values of controls and HD erythrocyte membranes were compared by a two-way analysis of variance²⁰ (Table 1). This two-tailed method of statistical analysis minimises possible fluctuations from day to day between separate experimental values which can often occur with biological samples. *P* is the significance of the difference of mean values of (*W/S*)_{control} and (*W/S*)_{HD} calculated using a two-way analysis of variance. The null hypothesis used was that HD membranes are not different in their physical state compared with that of normal controls. The difference of (*W/S*)_{control} and (*W/S*)_{HD} is significantly less than zero, suggesting an altered conformation and/or organisation of membrane proteins in erythrocytes in HD. In each of the separate experiments represented by the results in Table 1, (*W/S*)_{control} was less than (*W/S*)_{HD}. No alteration in sulphydryl group content of HD could be demonstrated within the sensitivity of the analytical method used²¹.



Fig. 1 Typical ESR spectrum of normal human erythrocyte membrane ghosts labelled with MAL-6. The amplitudes of the $M_I = +1$ weakly and strongly immobilised lines are indicated by *W* and *S*, respectively.

The *W/S* ratio was not altered from control values in erythrocyte ghosts from non-HD patients on diazepam. This parameter of MAL-6 was actually smaller than control values (3.2 compared with 3.7) in erythrocyte membranes from patients without HD who were on a butyrophenone and a phenothiazine. This change is in the opposite direction of the results in HD membranes (Table 1). Other workers have previously reported decreased *W/S* ratios of MAL-6 in bovine erythrocyte membranes on incubation with phenothiazine derivatives¹⁹. These findings together with the fact that some of our HD patients were on no medication, suggest that the increased values of *W/S* in HD (Table 1) are not simply due to drug effects. In addition, the clinical manifestations of HD in the patients in this study ranged from very early mild stages to severe, bed-ridden, late stages. Most were at home, active, and on a regular, normal diet. That the *W/S* ratio was increased in HD compared with normal controls in each experiment suggests that the current results are not due to effects of a particular stage of the disease, diet, or physical activity.

An alteration in the physical state of membrane proteins in erythrocytes in HD has been suggested by these experiments (Table 1). These results may be due to an altered enzyme or other membrane protein, altered amounts of membrane constituents, or altered constituent organisation. An additional possibility may be the presence of a membrane-bound moiety in HD, not found in controls. Experiments designed to discern these possibilities are in progress.

A difference in the conformation and/or organisation of membrane proteins in the basal ganglia like that found in erythrocytes in the present study may possibly give rise to the altered enzymatic behaviour observed in HD (refs 5, 6). Alternatively, the reduced activity of these enzymes in

Table 1 Comparison of ESR spectral amplitudes of MAL-6 attached to weakly immobilised SH groups (W) with that of MAL-6 attached to strongly immobilised SH groups (S) in control and Huntington's disease (HD) erythrocyte membranes

Mean $\pm$ s.e.m. $n = 10$	(W/S) _{control} 3.73 ± 0.11	(W/S) _{HD} 4.26 ± 0.11
	$P < 0.005$	

* P value calculated by a two-way analysis of variance using the null hypothesis that the respective means are equal.

HD may be a secondary consequence of an altered milieu in which they are located. The relationships of the present results to the molecular mechanisms by which the pathological and clinical manifestations of HD arise are unclear, but the alterations in the physical state of erythrocyte membranes which are outside the central nervous system suggest that this disease may be associated with a general membrane abnormality.

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Marked enhancement of interferon production in 5-bromodeoxyuridine treated human lymphoblastoid cells

MANY human lymphoblastoid cells produce low levels of interferon spontaneously¹⁻⁵ and this has been attributed to the presence of the Epstein-Barr virus (EBV) in such cells⁶⁻⁷. No clear relationship has been established, however, between the presence of EBV and spontaneous interferon production⁸. Since the latent EBV genome contained in human lymphoblastoid cells can be activated by treatment of these cells with halogenated pyrimidines^{9,10} or inhibitors of protein synthesis¹¹ we determined the effect of 5-bromodeoxyuridine (BrdU) on interferon production in these cells in relation

to the induction of EBV. Previous studies on the effect of halogenated pyrimidines on interferon production have yielded conflicting results ranging from no effect¹²⁻¹⁴ to inhibition¹⁵, or slight stimulation^{15,16}. We report here that BrdU markedly increases both spontaneous and virus-induced interferon production in three human lymphoblastoid cell lines, without any apparent correlation with EBV induction.

The four human lymphoblastoid cell lines studied were chosen for their different characteristics in respect of origin, expression of EBV specified antigens, susceptibility to BrdU induction, and the capacity to produce interferon spontaneously. As reported by others^{4,5}, we found that Namalwa cells produce low levels of interferon spontaneously when maintained at a concentration of 10^7 cells per ml for 24 h (Table 1). Pretreatment of exponentially multiplying cultures of Namalwa cells with BrdU markedly increased the titre of autogenous interferon produced. Maximum titres of spontaneous interferon (160-320 reference units per ml) were obtained after 72 h treatment with BrdU $25 \mu\text{g ml}^{-1}$ (Table 1). (No loss of viability was observed in BrdU treated cultures although the rate of cell multiplication was reduced.)

The spontaneous interferon produced in BrdU-treated cultures of Namalwa cells fulfilled the usual criteria for acceptance of a viral inhibitor as an interferon¹⁷ and was neutralised by a sheep anti-human leukocyte interferon serum. Spontaneous interferon production by Namalwa cells seemed to occur in the absence of EBV induction since no early antigen (EA) or virus capsid antigen (VCA) could be detected by immunofluorescence in either control or BrdU-treated cultures, in accord with other reports^{18,19}.

Raji cells, however, do express EA after treatment with BrdU (refs 17, 18), but have been reported not to produce interferon spontaneously^{2,4,5}. Similarly, we found that no spontaneous interferon was produced in untreated cultures of Raji cells even after 100-fold concentration of the culture supernatants (Table 1). Spontaneous interferon was detected in cultures of Raji cells after 72 h treatment with BrdU, however (Table 1). The spontaneous interferon was neutralised by a sheep antihuman leukocyte interferon serum.

BrdU also increased the amount of interferon produced by Namalwa and Raji cells in response to induction with Newcastle disease virus (NDV). Maximum titres were obtained after either 72 h treatment with BrdU $25 \mu\text{g ml}^{-1}$ or after 24 h treatment with BrdU $100 \mu\text{g ml}^{-1}$ (Table 2). (Treatment with BrdU $100 \mu\text{g ml}^{-1}$ for more than 24 h resulted in considerable cell toxicity.) Treatment of Namalwa cells with BrdU increased, by up to 32-fold, the titre of interferon produced on induction with NDV. A median increase of 8-fold in interferon titre was obtained in a total of 13 determinations (Table 2). Similarly, treatment of Raji cells with BrdU for 48-72 h gave a median increase of 33-fold in the titre of virus-induced interferon production (Table 2). The titres of both spontaneous and virus-induced interferon from BrdU treated cultures remained at the same high levels even when treated cells were subsequently cultivated in the absence of BrdU for 72 h before interferon induction. The continued presence of BrdU during the period of interferon induction did not affect the titre of either spontaneous or virus induced interferon obtained (experiment 4, Table 2). Treatment of Namalwa cells with 5-iododeoxyuridine (IrdU) gave a similar increase to that obtained with BrdU, in the titre of both spontaneous and virus-induced interferon, even though IrdU is a more efficient inducer of latent virus^{19,20}.

Although the mechanism of the enhancement of interferon production by BrdU is unknown, potentiation of interferon production is maximal after prolonged treatment of exponentially multiplying cultures with BrdU, conditions which would be expected to result in incorporation of BrdU into cellular DNA. In addition, the enhancement of

Table 1 Effect of BrdU on spontaneous interferon production and early antigen expression in human lymphoblastoid cells

Cell line	Time of treatment (h)	Interferon titre* (reference units per ml)		Frequency of EA-positive cells per 50,000	
		Control	BrdU treated	Control	BrdU treated
Namalwa Experiment 1	5	15	40	0	0
	24	10	40	0	0
	48	20	80	0	0
	72	10	160	0	0
Raji Experiment 2	72	15	320	0	0
BJAB Experiment 1	26	< 5	< 5	4	79
	48	< 5	< 5	3	246
	72	< 5	30	1	111
	72	< 10†	240†	1	146
BJAB Experiment 2‡	24	< 5	< 5	0	0
	24	< 10	120	0	0

Lymphoblastoid cells were cultivated in RPMI 1640 medium with 10% foetal calf serum (GIBCO) in stirred suspension culture, for the times indicated, with or without BrdU ($25 \mu\text{g ml}^{-1}$). Each culture was then centrifuged (800g, 15 min), washed once, and the cells resuspended at a concentration of 10^7 cells per ml in the same medium but without BrdU. After 24 h incubation at 37°C in stirred suspension culture, the culture supernatant was harvested by centrifugation (800g, 15 min) and assayed for interferon content after 10-fold concentration with Aquacide (Calbiochem) and dialysis against phosphate buffered saline, pH 7.4. EBV early antigen (EA) was determined on cells at the same time as the culture supernatants were harvested for the assay of interferon. EA was determined on acetone-fixed smears by indirect immunofluorescence using human sera and fluorescein-conjugated goat anti-human IgG globulin (Huntingdon Laboratories, U.S.A.).

Virus-induced interferon production was determined in the same manner as described in Table 1 with the exception that NDV was added to the culture at the concentration indicated. All samples were dialysed at pH 2.0 for 7 d to inactivate residual NDV before assay for interferon.

*Interferon was assayed by inhibition of the cytopathic effect of 100 TCID₅₀ of vesicular stomatitis virus on monolayer cultures of human foreskin fibroblasts as previously described³². Interferon titre expressed as the reciprocal. All interferon titres are expressed in terms of the international reference preparation 69/19 (National Institute of Medical Research, London).

†Sample concentrated 100-fold.

‡To minimise toxicity and increase efficiency of incorporation into DNA, BrdU was added to a culture already multiplying exponentially. Samples concentrated 50-fold.

interferon production by BrdU was reduced when cells were treated with BrdU in the presence of added thymidine, or when BrdU treated cells were exposed to visible light. Incorporation of BrdU into cellular DNA is also known to be necessary for BrdU activation of EBV (refs 20–22). It seems unlikely, however, that EBV induction is responsible for the increase in either spontaneous or virus-induced interferon production in BrdU treated cultures, since this enhancement occurred in Namalwa cells in the absence of EBV induction. Namalwa cells, however, do contain two (probably incomplete) copies of the EBV genome²³. Therefore to exclude the possible involvement of EBV induction we investigated the effect of BrdU on interferon production in the human lymphoblastoid cell line BJAB which does not express the EBV-specified nuclear antigen EBNA and which is negative for EBV by nucleic acid hybridisation²⁴. Spontaneous inter-

feron was detected (after 50-fold concentration) in the culture supernatants from BrdU treated BJAB cells, but not in the supernatants from untreated control cultures (Table 1). The spontaneous interferon produced was neutralised by a sheep anti-human leukocyte interferon serum. There was also a clear cut increase in NDV-induced interferon production in BrdU treated BJAB cells (Table 2). No EA or VCA was detected either before or after treatment of BJAB cells with BrdU. These results strongly suggest that induction of EBV by BrdU is not responsible for the observed increase in interferon production in BrdU treated cultures.

Namalwa, Raji and BJAB are all B-lymphocyte derived cell lines^{18,24}. It was therefore of interest to determine the effect of BrdU on the T-lymphocyte cell line JM²⁵. The JM cell line did not produce interferon spontaneously either

Table 2 Effect of BrdU on virus-induced interferon production in human lymphoblastoid cells

Cell line	Time of treatment (h)	NDV concentration (HA units/ml)	Interferon titre* (reference units per ml)	
			Control	BrdU treated ($25 \mu\text{g ml}^{-1}$)
Namalwa	Experiment 1	256	1,280	19,200†
	Experiment 2	256	3,200	25,600
	Experiment 3	256	800	25,600
	Experiment 4	25.6	1,600	9,600
		256	2,400	19,200
		256‡	3,200	38,400
		1,280	9,600	19,200
Raji	Experiment 1	256	60	240
		256	320	4,800
		256	320	9,600
		256	240	8,600
BJAB	Experiment 1	256	< 20	480
	Experiment 2	256	40	160

*Titre expressed as the reciprocal.

†Culture treated with BrdU $100 \mu\text{g ml}^{-1}$.

‡Culture not washed after removal of BrdU.

before or after treatment with BrdU. In addition, treatment of JM cell with BrdU ($25 \mu\text{g ml}^{-1}$) decreased the titre of interferon produced in response to induction with NDV. It remains to be determined whether the behaviour of JM cells is characteristic of T cells in general. Preliminary results suggest that BrdU also increases NDV-induced interferon production in a non-lymphoid Moloney sarcoma virus transformed mouse cell line (C 243-3 cells), but not in a line of human or rat fibroblasts.

BrdU uniformly incorporated into cellular DNA has been reported both to increase²⁶ and to selectively inhibit²⁷ the transcription of certain genes in mammalian cells. Interferon induction is sensitive to actinomycin D²⁸ and represents a process analogous to enzyme induction²⁹. Two possible explanations of our results would be that either BrdU increases the transcription of the interferon messenger or inhibits the transcription of an inhibitor of interferon synthesis.

Human lymphoblastoid cells (and in particular the Namalwa cell line) have received attention as a source of human interferon for clinical trials^{30,31}, since these cells can be cultivated in suspension culture in virtually limitless quantity.

Our results show that up to a 32-fold increase in interferon titre can be obtained by treatment of Namalwa cells with BrdU. Furthermore, higher interferon titres can be obtained from BrdU-treated cultures of Namalwa cells, inoculated with considerably less virus inducer than from untreated cultures induced with optimal amounts of NDV (Table 2, experiment 4). We believe that these results may therefore be of considerable importance for the large scale production of human interferon.

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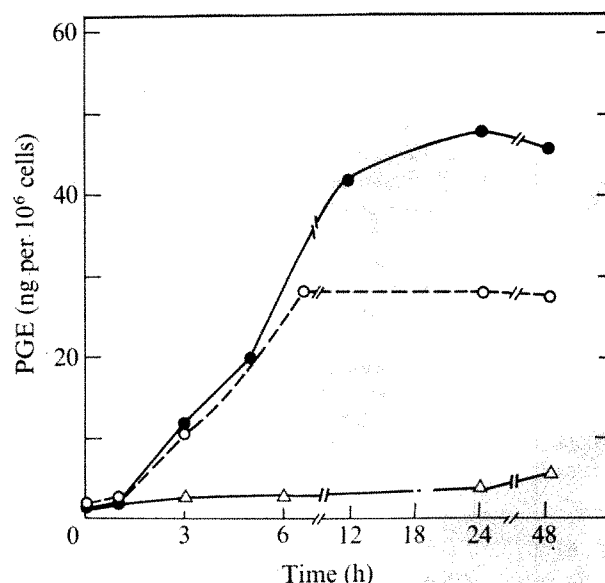
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Stimulation of prostaglandin E production in cultured human fibroblasts by poly(I) · poly(C) and human interferon

PROSTAGLANDINS (PGs) have emerged as one of the principal mediators of the inflammatory process¹⁻³. Elevation of tissue concentration of PGs was noted following inflammation^{3,4}, or treatment with various hormones^{5,6}, cyclic nucleotides (ref. 7 and U.Z., B. Strulovici and H.R.L., in preparation), serum⁸, drugs^{8,9} and antigen¹⁰. The beneficial effects of anti-inflammatory drugs have been correlated with their ability to suppress PG production either by curtailing precursor availability (glucocorticosteroids^{11,12}) or by inhibiting PG synthetase activity (non-steroidal drugs¹). Viral infections are associated with interferon production¹³ and with inflammation¹⁴. Little is known about the possible involvement of interferon in the inflammatory reaction, though it has been suggested that interferon may have anti-inflammatory properties¹⁵. It has been shown that an interferon inducer, double-stranded polyinosinate-polycytidylic acid [poly(I)·poly(C)], as well as human interferon, stimulate hyaluronic acid production by human synovial fibroblasts in culture¹⁶. Overproduction of hyaluronic acid is known to be associated with the inflammatory process¹⁴; the effect is prevented by cortisol or indomethacin¹⁶. We report here the stimulation by poly(I)·poly(C) and by human interferon of prostaglandin E (PGE) production by human synovial and foreskin fibroblasts in culture. Cortisol inhibited this effect.

Fibroblasts derived from synovial membranes of patients undergoing surgery for meniscus tear were grown in culture using methods described by Castor *et al.*¹⁷. Human fibroblast interferon (2×10^4 U ml⁻¹) was prepared from foreskin fibro-

Fig. 1 Time course of stimulation of PGE production in cultured synovial fibroblasts by poly(I)·poly(C) and human interferon. Synovial fibroblasts were sown (about 4×10^5 cells per ml) in a medium containing Parker CMRL-1066 (80%), heat-inactivated human serum (10%) and foetal calf serum (10%), supplemented by L-glutamine, penicillin and streptomycin, and grown as monolayers. Samples of the medium were withdrawn after the time-intervals indicated for PGE determination. Δ , Controls; $\bullet$, poly(I)·poly(C) added to the medium ($100 \mu\text{g ml}^{-1}$); $\circ$, human fibroblast interferon added (100 U ml⁻¹).



blasts (FS4) by the method of Havell and Vilcek¹⁸. Partially purified human lymphoblastoid interferon (purification about 200-fold) was prepared as described by Anfinson *et al.*¹⁹. Poly(I)·poly(C) (Miles) or interferon were incubated with the synovial fibroblast and foreskin cell cultures for various times and the accumulation of prostaglandins of the E group in the medium was determined by radioimmunoassay as previously described²⁰.

Both poly(I)·poly(C) ($100 \mu\text{g ml}^{-1}$) and human interferon (100 U ml^{-1}) stimulated PGE production by synovial fibroblasts: increased accumulation of PGE in the medium was noted within 3 h and a peak was reached at 24 h (Fig. 1). Mouse interferon did not stimulate PGE production in these human cells. The lowest effective concentration of poly(I)·poly(C) was $10 \mu\text{g ml}^{-1}$ and the response was maximal at $20\text{--}100 \mu\text{g ml}^{-1}$ (Fig. 2). Single-stranded polyinosinate (poly I) or polycytidylate (poly C) or polyadenylate-polyuridylylate [poly(A)·poly(U)] did not stimulate PGE production when added to cultured synovial fibroblasts at $100 \mu\text{g ml}^{-1}$. Poly(I)·poly(C) did not induce PGE production

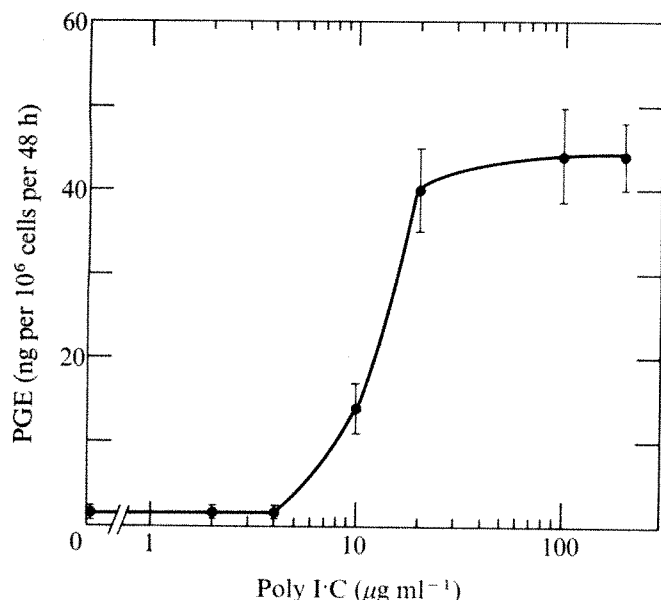


Fig. 2 Dose-response curve of stimulation of PGE production in cultured human synovial fibroblasts by poly(I)·poly(C). PGE concentration in the medium was determined by radioimmunoassay. Vertical bars, s.e.m. ($n = 8$). For experimental details see Fig. 1 legend.

in cultured human peripheral blood lymphocytes, which are known not to respond to double-stranded RNA with interferon production. In contrast, human interferon and poly(I)·poly(C) stimulated PGE production in two lines of human foreskin fibroblasts tested (FS7 and FS4). Stimulation was proportional to the amount of interferon added (Fig. 3). Both fibroblast and lymphoblastoid human interferon stimulated PGE production in foreskin cells. Addition of partially purified lymphoblastoid interferon to the medium (100 U ml^{-1}) elicited a 40-fold stimulation of PGE. Cycloheximide ($10 \mu\text{g ml}^{-1}$) and actinomycin D ($2 \mu\text{g ml}^{-1}$) prevented the stimulation by poly(I)·poly(C) of PGE formation (data not shown). The stimulatory action of poly(I)·poly(C) and of interferon on PGE production by foreskin fibroblast cultures was also abolished by concomitant addition of cortisol ($2 \mu\text{g ml}^{-1}$) to the medium (Fig. 4). Both poly(I)·poly(C) and human interferon had a moderate (30%) inhibitory effect on synovial fibroblast proliferation and cortisol prevented this effect (data not shown).

This is the first report to show that interferon is capable

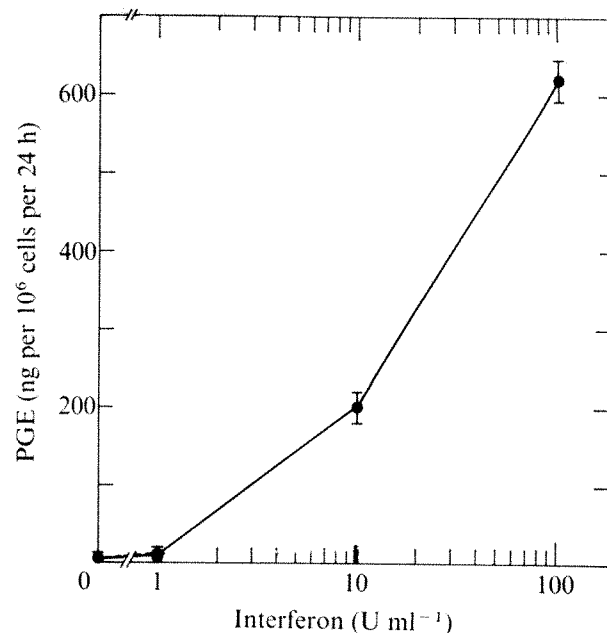
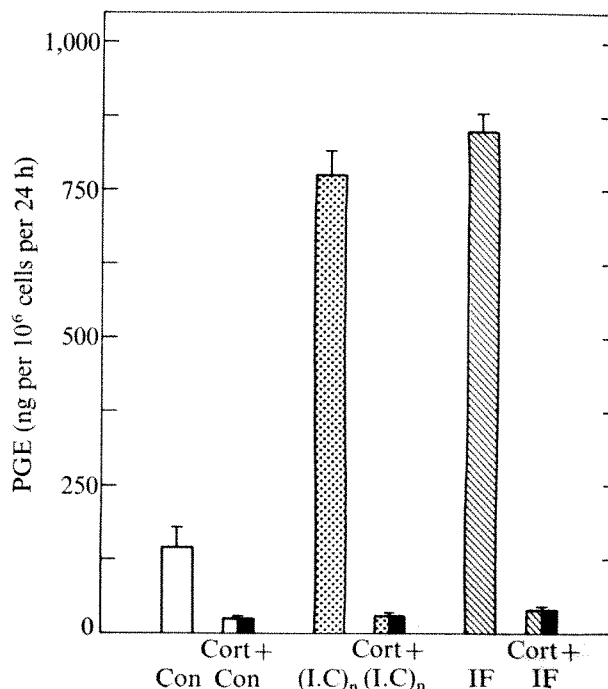


Fig. 3 Dose-response curve of stimulation of PGE production by human foreskin fibroblast interferon in human foreskin fibroblast cultures. The cells (2×10^6 cells per ml) were sown in Eagle's MEM medium containing 5% heat-inactivated foetal calf serum and penicillin and streptomycin and incubated for 24 h at 37°C with various doses of interferon. PGE was determined by RIA. Bars represent mean $\pm$ s.e.m.

of stimulating the production of PGE, an established mediator of inflammation¹⁻³. This effect shares the known species specificity of interferon action, since only interferons of human origin, but not mouse interferon, induced PGE synthesis in human fibroblast cells. The efficacy of partially purified lymphoblastoid interferon makes it unlikely that the observed effect was due to contaminating

Fig. 4 Prevention by cortisol (Cort; $2 \mu\text{g ml}^{-1}$) of the stimulatory effect of poly(I)·poly(C) [(I.C.)_n; $100 \mu\text{g ml}^{-1}$] and human fibroblast interferon (IF; 100 U ml^{-1}) on PGE production by human foreskin fibroblasts. Con, control. Other experimental details as for Fig. 3.



material. This study shows that poly(I)-poly(C), which induces interferon formation in synovial and foreskin fibroblasts¹⁶⁻¹⁸, is also capable of stimulating PGE production. Poly(A)-poly(U) and single-stranded poly(I) or poly(C), which do not induce interferon formation, are not active in this respect. The induction of PGE synthesis by either interferon or poly(I)-poly(C) has a latency of about 3 h. During this time both RNA and protein synthesis are required.

Cortisol, a well known anti-inflammatory drug, which is able to reduce PGE production by inflamed synovial membrane^{12,21}, abolished PGE production induced by both interferon and poly(I)-poly(C). We suggest that interferon may have a role in propagating the inflammatory effect of viral infections through prostaglandins, which have been shown to participate in the body's general defence mechanism²². It is of interest that both glucocorticoids and aspirin seem to enhance the pathogenic effects of viral infections^{23,24}. The present findings are difficult to reconcile with those of Koltai and Mecs¹⁶, who have attributed to the interferon inducer, poly(I)-poly(C), an inhibitory effect on inflammation.

Another common effect of interferon and prostaglandins of the E type is their potent inhibition of the replication of several cell types²⁵⁻²⁷. Cortisol and indomethacin, which inhibit PGE production^{1,11,12,21}, stimulated tumour cell replication²⁸ and prevented the inhibitory effect of interferon on synovial fibroblast growth. The significance of prostaglandin synthesis in the mode of action of poly(I)-poly(C) and interferon as antiviral and anti-tumour agents is not yet clear and is under further study.

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Virus-like particles from both control and scrapie-affected mouse brain

CHO and Grieg¹ reported that 14-nm virus-like particles could be extracted from the brains of mice in the late clinical stage of scrapie but not from the brains of young, uninoculated mice. The extraction procedure involved repeated fluorocarbon treatment followed by equilibrium banding on CsCl gradients. One band was obtained from extracts of normal brain at a density of 1.29-1.30 g cm⁻³ and two bands were obtained from scrapie brain extracts at densities of 1.29-1.30 g cm⁻³ and 1.33-1.34 g cm⁻³, respectively. Electron microscope studies showed that 14-nm particles were found mainly in the 1.33-1.34 g cm⁻³ fraction derived from scrapie brains. It was subsequently reported² that similar particles could be obtained at the same density after centrifuging extracts of scrapie hamster brain. This second study also showed that the exhaustive extraction procedure resulted in a large loss of infectivity, but the small amount of infectivity remaining sedimented mainly in the same position as the 14-nm particles. From these observations it was suggested that the 14-nm particles might be the causative agent of scrapie. Similar studies have been carried out at Compton during the last year in an attempt to repeat these findings with brain both from mice in the late clinical stage of infection and from sheep with natural scrapie. In none of these studies was a visible band seen at 1.33-1.34 g cm⁻³ after isopycnic centrifugation in CsCl. Electron microscopy studies of this fraction derived from scrapie mice showed 14-nm particles, resembling those originally reported, in one field only; the sample was rich in other particulate material common to control samples also. In order to resolve these different findings a collaborative study was arranged at Lethbridge in which scrapie mouse brain and brains of age-matched control mice (previously injected with normal mouse brain) from both Institutes were extracted. We report here that 14-nm particles can be visualised from both control and scrapie-affected mouse brain.

Four groups of mice were used. Two groups of female, random bred, Swiss white mice of the Animal Diseases Research Institute (Western) Lethbridge were inoculated intracerebrally with 0.03 ml of a 1% (wet weight) suspension of mouse brain in physiological saline; the control group was injected with a suspension of normal mouse brain and the scrapie group with a suspension of brain from clinically-affected mice. The other two groups were specific pathogen free, female, Compton white mice injected in an identical manner. The Lethbridge mice were infected with the Compton strain of mouse-adapted scrapie obtained from Dr W. J. Hadlow³ and the Compton mice were infected with the same strain⁴, passaged at Compton. Scrapie mice were killed in the late clinical stage of the disease (after 18 weeks) and the brains collected and stored frozen (-20 °C). Control brains were collected at the same time. The brain samples from Compton were coded by an independent observer before being taken to Lethbridge. The weights of the various brain pools used for extraction were as follows: 97 g of control brain and 80 g of scrapie brain from Compton, 123 g of control brain and 175 g of scrapie brain from Lethbridge. The method of extraction used was the same as originally described¹. After the extensive Freon extraction procedure, material equivalent to 12 g of original brain in a volume of 1.0 ml was centrifuged on a CsCl gradient in a Spinco 50.1 Ti Sw Rotor at 243,000g for 72 h.

After centrifugation of the Lethbridge control sample only one distinct band was seen in the CsCl gradient, at a density of 1.29-1.30 g cm⁻³, whereas the Lethbridge scrapie sample gave two bands, one identical to the control and an additional, faint band which was seen at a density of

1.33–1.34 g cm⁻³. Centrifugation of both Compton samples (normal and scrapie) gave less distinct bands with most of the material sedimenting to a density of 1.29–1.30 g cm⁻³. Ten equal fractions of 0.45 ml were collected from all four samples by syringes from the side of the tube and each fraction was subsequently examined under the electron microscope as described before¹ at both Compton and Lethbridge.

At Lethbridge spherical particles similar to those originally described by Cho and Greig¹ were seen at a density of 1.33–1.34 g cm⁻³ from both the control and both the scrapie extracts. The particles were morphologically similar from all four sources. At Compton very few particles were seen in the projected size range in any of the four samples; however, conditions of storage may have accounted to some extent for these different observations at the two centres. The evidence indicated that, where particles were seen, they were present in both scrapie and control preparations.

These findings suggest that the particles were either of host origin or, alternatively, represent an unknown species of virus which is present in both normal and scrapie-affected mice. The former possibility is considered more likely since similar particles have also been obtained from the brains of scrapie-affected hamsters² and goats (H.J.C. unpublished).

Since 14-nm particles can be obtained from both control and scrapie-affected brain, it seems that they are not specifically associated with scrapie. The scrapie infectivity reported to be present at a density of 1.33–1.34 g cm⁻³ may therefore be associated with some entity other than the 14-nm particles. Alternatively, it is possible that although the particles obtained from both control and scrapie mice were morphologically similar, there may be biochemical differences; for example the 14-nm particles from scrapie brain might harbour a scrapie-specific nucleic acid. This possibility can be tested by a detailed analysis of the biochemical components present in highly purified preparations of 14-nm particles from several sources. Some preliminary, immunological studies have already been carried out involving treatment of 14-nm particles from scrapie mouse brain with anti-mouse ferritin serum prepared in rabbits. Aggregation of the 14-nm particles was observed by electron microscopy indicating that the particles isolated from scrapie brain share common antigen(s) with mouse ferritin (H.J.C. and Darcel, unpublished).

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Role for nucleocapsid protein phosphorylation in the transcription of influenza virus genome

LIKE other minus-strand RNA viruses^{1–3}, influenza virus seemed to contain protein kinase as well as viral transcriptase in, or in association with, nucleocapsid structure⁴. The kinase phosphorylates NP-protein constituting the nucleocapsid, but none of the other virion proteins in *in vitro* reaction⁵. Since the influenza virus RNA serves as a template for transcription in the form of nucleocapsid in which the viral RNA is tightly packed in helical array of NP-protein^{7–9}, it is tempting to implicate the NP-protein phosphorylation in the control of viral transcription. We report here that the phosphorylation of NP-protein stimulates viral transcription, and that some conformational changes in the nucleocapsid may be involved in the stimulation of transcription.

Influenza A₂ virus (R1/57/57) was grown in embryonated eggs and purified as described previously⁶. None of the viral proteins was phosphorylated to a significant extent during virus growth in eggs⁶. The purified virus suspension was divided into two portions, one of which was phosphorylated with the endogenous protein kinase as described (legend to Fig. 1). In these conditions, only NP-protein of the virion proteins was phosphorylated and its phosphate-accepting sites were saturated within 20 min (ref. 6). The phosphorylated nucleocapsid was then isolated and made free of ATP (legend to Fig. 1). Since it was crucially important to confirm complete removal of ATP from the nucleocapsid, the other portion of the nucleocapsid preparation was treated in the same manner as the first, except that ATP was added at the end of incubation and then removed as above. This unphosphorylated preparation will be referred to as 'native nucleocapsid'. A separate experiment in which ¹⁴C-ATP was incubated with the nucleocapsid and then removed by the same procedure, showed that a residual amount of free ATP, if any, was less than 10⁻⁶ of the input amount, which for our purposes was negligible. The nucleocapsid preparations thus obtained were practically free of neuraminidase and haemagglutinin as determined by gel electrophoresis, but contained a considerable amount of matrix (M) protein and a few minor components (one of which may correspond to viral transcriptase), in addition to NP-protein.

A comparison of circular dichroism (CD) spectra of the native and the phosphorylated nucleocapsid is given in Fig. 1a. Phosphorylation caused a significant reduction in negative ellipticity in the range 200–250 nm, suggesting the reduction of secondary structure content in the protein moiety of nucleocapsid. This conformational change seemed reversible. Thus, the treatment of phosphorylated nucleocapsid with protein phosphatase¹⁰ could remove 80% of the phosphate which had been introduced by the *in vitro* phosphorylation and the CD spectrum of the dephosphorylated nucleocapsid was indistinguishable from that of native one (Fig. 2b). The phosphorylation-induced conformational changes were also reflected in the increased heat lability of the nucleocapsid (insert Fig. 1).

To determine the effect of protein phosphorylation on the nucleocapsid-directed transcription, transcription had to take place in the absence of phosphate donor for protein kinase¹¹ and in the present study this was accomplished by using an ATP analogue, β - γ -imido-ATP or AMPPNP (ref. 11). This analogue can be used, although less efficiently, as a substitute for ATP for the viral transcription, but it will not serve as phosphate donor for protein kinases¹¹.

When the native or phosphorylated nucleocapsid was incubated in the ordinary reaction mixture for transcription

(ATP present), the kinetics of the transcription were similar to each other, indicating that the transcriptional activities of both the preparations were equally preserved during the preceding treatment (Fig. 2). In contrast, when transcriptional activities were assayed in a reaction mixture in which ATP was replaced by AMPPNP and therefore free of phosphate donor for protein kinase, the native nucleocapsid exhibited two-phase kinetics of transcription; the reaction rate was significantly low for the first 3 h and then increased. This was observed consistently in six separate

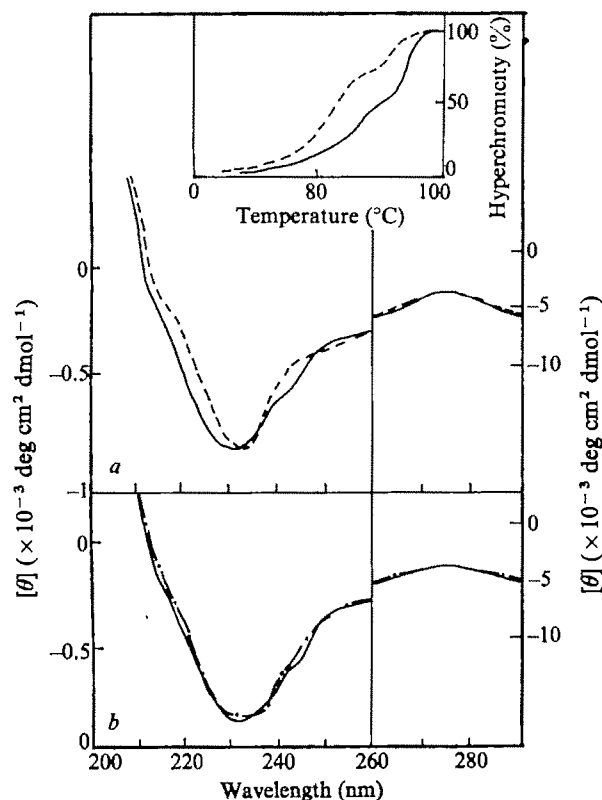


Fig. 1 Comparison of the native and phosphorylated nucleocapsids in CD spectra and in denaturation temperatures. *a*, Purified influenza A₂ virions (3.2 mg protein) were incubated in 3 ml of the mixture for phosphorylation reaction containing 0.1 mM ATP, 8 mM Mg²⁺, 10 mM dithiothreitol (DTT), 0.1% Nonidet-P40 and 10 mM Tris-Cl (pH 8.0) at 32 °C for 30 min. The mixture was cooled in ice and overlayed on a 10% sucrose solution containing 25 mM Tris-Cl (pH 8.0), 1 mM Mg²⁺, and 0.5 mM DTT, and centrifuged at 4 °C and 100,000g for 100 min to pellet the nucleocapsid. The pellet was resuspended and pelleted again through the sucrose solution to remove residual ATP completely. The final nucleocapsid pellet was resuspended in 25 mM Tris-Cl (pH 7.4) to give 0.35 mg protein ml⁻¹ and subjected to the CD spectra determination at 20 °C. The native nucleocapsid was prepared similarly except that ATP was added after the incubation and then removed as above. CD spectra were determined in a Jasco J-20 automatic spectropolarimeter. —, Native nucleocapsid; ---, phosphorylated nucleocapsid. In the range 210–260 nm, the mean ellipticity θ is expressed in deg cm² dmol⁻¹ of amino acid residue (assuming a mean molecular weight of an amino acid as 103) and in the range 260–290 nm deg cm² dmol⁻¹ of nucleotide residue (assuming a mean molecular weight of a nucleotide as 330). *b*, The phosphorylated nucleocapsid described in *a* (2.0 mg protein) was incubated in 5 ml of a mixture containing 10 mM sodium acetate, pH 5.8, 5 mM L-ascorbic acid and 125 μ g protein of partially purified protein phosphatase from bovine spleen¹⁰ at 37 °C for 1 h. The nucleocapsid was then pelleted by sedimentation through the sucrose solution as above and resuspended in 25 mM Tris-Cl (pH 7.4) to give 0.35 mg protein ml⁻¹ for the CD spectrum determination. —, Native nucleocapsid; ---, dephosphorylated nucleocapsid. Inset, Hyperchromicity determinations at 260 nm during temperature rise at 0.5 °C min⁻¹.

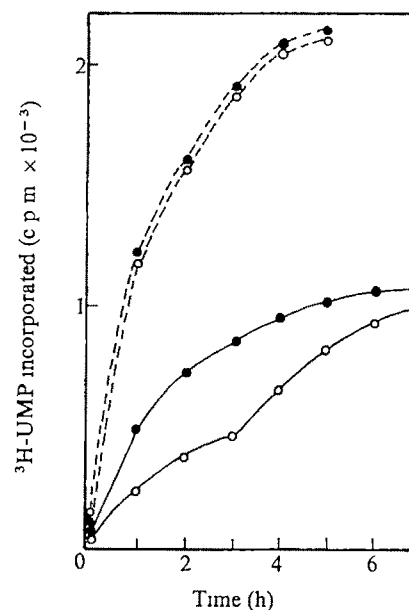


Fig. 2 Transcriptional activities of the native and phosphorylated nucleocapsids in the presence or absence of phosphate donor. The reaction mixture (lacking phosphate donor) contained, in 1.0 ml, 0.1 mM each of AMPPNP, CTP and GTP, 0.2 μ M (20 μ Ci) of ³H-UTP, 8 mM Mg²⁺, 1 mM Mn²⁺, 80 mM NaCl, 5 mM DTT, 10 mM Tris-Cl (pH 8.2) and 2.4 mg protein of the influenza virus nucleocapsid; and was incubated at 33 °C. Aliquots were taken in duplicate at the times indicated and the trichloroacetic acid-insoluble radioactivities were determined in a scintillation spectrometer. In another reaction mixture (containing phosphate donor), AMPPNP was replaced by 0.1 mM of ATP. ●, Phosphorylated nucleocapsid; ○, native nucleocapsid; —, with AMPPNP as substrate; ---, with ATP as substrate.

experiments with different preparations of native nucleocapsid, but such reaction lag was not seen with the several preparations of phosphorylated nucleocapsid. We interpret the results as indicating that the phosphorylation of NP-protein leads to the stimulation of viral transcription. It is conceivable that the conformational change induced by the phosphorylation is involved in the stimulation mechanism. Such stimulation by the previous phosphorylation of nucleocapsid was undetectable in the ordinary transcription system containing ATP, presumably because the phosphate-accepting sites of NP-protein were rapidly saturated in the beginning of transcription reaction⁹.

To examine whether the late recovery of transcription with the native nucleocapsid in the absence of ATP resulted from very slow phosphorylation of the nucleocapsid using CTP, UPT or GTP present, the CD spectra of native nucleocapsid were measured at various times during the transcription. The CD spectrum change below 250 nm which was characteristic to the phosphorylation of nucleocapsid (Fig. 1) was not seen; however, instead, a marked ellipticity change was observed in the range 270–280 nm at about 3 h of incubation (spectra not shown). The ellipticity change at 273 nm as a function of incubation time was plotted (Fig. 3) and the results indicated that the nucleocapsid underwent conformational rearrangement (possibly the changes in RNA-protein interaction) which was distinct from the conformational change caused by phosphorylation of NP-protein, and that the time of rearrangement coincided with the start of late stimulation of transcription. Since this rearrangement also occurred in the absence of nucleotidetriphosphates (Fig. 3c), it was neither the consequence of transcription nor of phosphorylation. Although it is

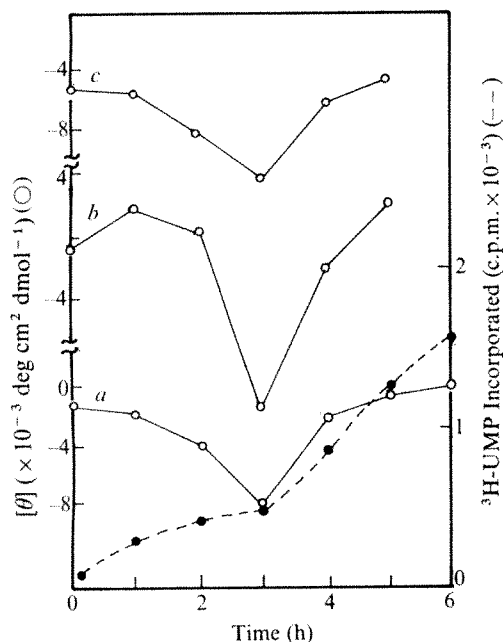


Fig. 3 The ellipticity changes at 273 nm during the transcription with native nucleocapsid. The native nucleocapsid (1.1 mg) was incubated in a CD measuring cell at 33 °C in a final volume of 1.3 ml of the reaction mixture for transcription (lacking phosphate donor) described in Fig. 2. The concentrations of AMPPNP, GTP and CTP were reduced to 0.01 mM so that the CD spectra could be read directly during the incubation. At the times indicated 20 μ l aliquots were withdrawn for the determination of ³H-UTP incorporation into the trichloroacetic acid-insoluble fraction (transcription, — — —), immediately after which the CD spectra were determined and the ellipticities at 273 nm were plotted (a), — — —. Each CD measurement took about 10 min and this time was not included in the total incubation time. b, The ellipticity change at 273 nm incubated in the reaction mixture which contained ATP instead of AMPPNP. c, The ellipticity change at 273 nm incubated in the reaction mixture from which all the nucleotidetriphosphates were omitted.

unknown at present whether this conformational rearrangement is an artefact due to the long incubation of isolated nucleocapsid, the result supports the concept that the native influenza virus nucleocapsid must undergo some conformational changes in order to be transcribed efficiently. This effect is not observed, however, in the ordinary transcription reaction using ATP, or in the reaction with prephosphorylated nucleocapsid using AMPPNP because the transcription is stimulated more effectively by the phosphorylation of nucleocapsid with the endogenous protein kinase.

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3' Terminal sequences of 16S rRNA do not explain translational specificity differences between *E. coli* and *B. stearothermophilus* ribosomes

DIRECT Watson-Crick base pairing between the 3' terminus of 16S RNA and messenger RNA seems to contribute to the recognition and binding of protein synthesis initiator regions by *Escherichia coli*^{1,2}. Since bacterial species differ in the pyrimidine-rich sequences at the 3' termini of their 16S RNAs, it has been suggested that this mRNA-rRNA interaction can account for observed differences in translational specificity among prokaryotes³. To test this hypothesis, we have asked whether it explains the choice of sites bound by *Bacillus stearothermophilus* ribosomes in two mRNAs—the R17 and Q β phage genomes. The RNA sequence data presented here lead to two conclusions. First, *B. stearothermophilus* ribosome recognition of two non-initiator Q β regions at high temperature⁴ is indeed correlated with an extensive mRNA-rRNA match. Second, surprisingly, the 3' terminal sequence of 16S rRNA from *B. stearothermophilus* is identical to that of *E. coli*⁵⁻⁷ except that the 3' terminal adenosine of *E. coli* rRNA is replaced by UCUA_{OH} in the thermophile. Thus, rRNA sequences at the 3' end of 16S rRNA cannot explain differences in the capacity of ribosomes from these two species to recognise RNA phage initiator regions at lower temperatures^{4,8,9}.

The translational specificity of ribosomes from *B. stearothermophilus* has previously been studied both at elevated temperatures (60-70 °C), where protein synthesis in this organism is optimal, and at a lower temperature (49 °C) where these ribosomes can be directly compared with those of *E. coli*. At 65 °C *B. stearothermophilus* ribosomes bind efficiently to the A protein initiator region in R17 RNA, while discriminating against the other two phage cistrons^{4,10} (Table 1). In Q β RNA none of the three normal initiation sites is bound by *B. stearothermophilus* ribosomes at high temperature. Instead, two non-AUG- or GUG-containing regions, one of which is recovered as a 35-nucleotide fragment (the typical size of ribosome-protected regions) and the other as a simple purine-rich nonanucleotide, are selected⁴. At a lower temperature (49 °C), where *E. coli* ribosomes strongly bind all three initiator regions in R17 RNA, *B. stearothermophilus* ribosomes retain good binding of the R17 A site and recognise the R17 replicase initiator region only weakly¹¹. With Q β RNA at 49 °C, the coat and replicase initiators are bound, but with much lower efficiency than when *E. coli* ribosomes are used to protect the same RNA¹¹. In addition, the non-initiating Q β sites bound at 65 °C by *B. stearothermophilus* ribosomes are nearly absent at 49 °C (ref. 11 and M. L. Goldberg and J. A. S., unpublished). A reasonable explanation for their disappearance is masking by RNA secondary structure existing at this temperature. Evidence that secondary structure can indeed sequester potential ribosome binding sites comes from observations on the RNA bacteriophage RNAs, where either fragmentation¹²⁻¹⁴ or unfolding with formaldehyde^{15,16} increases ribosome recognition of both authentic and spurious initiation sites in these mRNAs.

We began by asking whether the sites in R17 and Q β RNA which are selected by *B. stearothermophilus* ribosomes at 65 °C are related to each other in having a particularly high degree of complementarity to the 3' terminal sequence of *B. stearothermophilus* 16S RNA. The partial sequence data previously available^{4,17,18} suggested that this might be the case, but resolution of ambiguities remaining in the sequence of the Q β non-initiating sites as well as extension of the *B. stearothermophilus* 16S

Table 1 Sites bound by *B. stearothermophilus* ribosomes

Binding temperature (°C)	Site	Sequence	Theoretical T_m of mRNA-rRNA complex (°C)	References	
				Ribosome binding data	Sequence data
65	R17 A	CCUAGGAGGUUUGACCUAUG	38	4	10
	Q β noninitiator <i>a</i>	CUGAAAGGGGAGAUUACUCG	29	4	This work, 4
	Q β noninitiator <i>b</i>	GGAAAGGAGC	29	4	This work
49	R17 A	CCUAGGAGGUUUGACCUAUG	38	11	10
	R17 replicase	AUGAGGAUUACCCAUG	-7	11	10
	Q β replicase	AACUAAGGAUGAAAUG	-7	11	14, 27
	Q β coat	AAACUUUGGGUCAUUUGAUGAUG	-25	11	28
	R17 coat	CAACCGGGGUUUGAAGCAUG	-46	4, 11	10
Not bound	16S 3' end	HOAUCU <u>U</u> UCCUCCACUAG 5'	—	—	This work, 17, 18

Data were obtained from ribosome-mRNA binding experiments carried out at 65 °C (ref. 4) or at 49 °C (ref. 11). The mRNA sequences represent only the 5' terminal portions (including the AUG initiator triplet) of the bound sites. They are arranged so that the regions complementary to 16S rRNA are aligned. Italics indicate that portion of the *B. stearothermophilus* 16S rRNA sequence which differs from *E. coli*. At each temperature, all sites bound are shown. Since the Q β A site was not recognised by *E. coli* ribosomes tested in parallel at 49 °C, it is presumed that this region was masked at this temperature (as it is at 37 °C (ref. 13)) and unavailable for binding by *B. stearothermophilus* ribosomes (M. L. Goldberg and J.A.S., unpublished). The melting temperatures (T_m) of the mRNA-rRNA complexes were calculated according to the rules for base stacking interactions and for the formation of double helical structures in bimolecular reactions (ref. 26 and R. C. Yuan, personal communication). For this analysis, the complexes were drawn without bulge loops since the thermodynamic parameters for loop structures are less accurately known than are those for structures without loops. The R17 coat site-rRNA complex can be drawn with a bulge loop in an alternative configuration whose calculated T_m is higher than the value shown. The fact that the calculated T_m values fall below the temperatures at which ribosome binding is actually observed indicates that other molecular interactions on the ribosome contribute greatly to the stability of mRNA-ribosome complexes. In the cases where the T_m values of isolated mRNA-colicin fragment complexes have been measured, there is good agreement between the theoretical and experimental values²¹.

terminal sequence was required to reach a definite conclusion. Accordingly, we determined the nucleotide sequences of the two polypurine tracts in the Q β non-initiators and the entire 3' terminal T1 oligonucleotide from *B. stearothermophilus* 16S RNA.

Since only the polypurine tracts from the Q β fragments were required for analysis, we avoided ribosome binding as an isolation procedure and instead took advantage of the large size and unique composition of these oligonucleotides. We isolated them directly from a total pancreatic ribonuclease digest of ³²P-labelled Q β RNA after fractionation in two dimensions (see legend to Fig. 1). The 3' terminal T1 oligonucleotide of *B. stearothermophilus* 16S rRNA, also ³²P-labelled, was isolated by selective adsorption to dihydroxyboryl cellulose¹⁹. Sequences of all three oligonucleotides were deduced primarily by partial digestion with spleen phosphodiesterase, as shown in Fig. 1. Additional data required to establish the sequence of the 16S terminus was obtained by standard techniques²⁰ as detailed in Table 2.

The sequences presented in Table 1 show that the two Q β non-initiator regions and the R17 A site do indeed exhibit extensive complementarity to *B. stearothermophilus* 16S rRNA. The Q β non-initiators (oligonucleotides *a* and *b* in Table 2) can pair with the 3' end of *B. stearothermophilus* 16S RNA to yield a structure which is nearly as stable as the complex formed between the R17 A site and the *B. stearothermophilus* 16S terminus. This latter complex is calculated to be identical in stability to the R17 A site-*E. coli* rRNA duplex, which has recently been isolated and characterised^{2,21}. In contrast, complexes between the *B. stearothermophilus* 16S terminus and other R17 and Q β initiators are theoretically much less stable (Table 1), a property which may explain their inability to be bound by thermophilic ribosomes at high temperatures.

On the other hand, Table 1 also demonstrates that species-related differences in the degree of mRNA-rRNA complementarity cannot explain the mRNA binding preferences of thermophilic ribosomes when *E. coli* and *B. stearothermophilus* ribosomes are compared at 49 °C.

Table 2 Sequence analysis of the 3' terminal T₁ fragment from *B. stearothermophilus* 16S RNA

Digestion with pancreatic RNase	Digestions used for identification	Molar yields
Products obtained		
AU	Alkaline hydrolysis	1
AC	Alkaline hydrolysis	1.0
C	Alkaline hydrolysis	4.8
U	Alkaline hydrolysis	4.6
Digestion with pancreatic RNase after carbodiimide (CMC) blocking		
AUC	Pancreatic RNase after de-blocking, alkaline hydrolysis	
AC	Pancreatic RNase after de-blocking, alkaline hydrolysis	
UC	Pancreatic RNase after de-blocking, alkaline hydrolysis	
C	Pancreatic RNase after de-blocking, alkaline hydrolysis	
U~ ₃ C*	Pancreatic RNase after de-blocking, alkaline hydrolysis	
Digestion with U ₂ RNase		
A	Alkaline hydrolysis	
UCA	Pancreatic RNase $\pm$ CMC, alkaline hydrolysis	
C(C~ ₄ , U~ ₅)A _{OH}	T ₂ RNase: Cp/Up = 0.93	
	Snake venom phosphodiesterase: pC/pA = 3.7	
	pU/pA = 5.2	
	pC/pU = 0.71	
	Pancreatic RNase + CMC: C	
	UC	
	U~ ₃ C*	

*The expected product, UA_{OH}, was not conclusively identified in these digests, presumably because it was obscured by the streaking of U₃C.

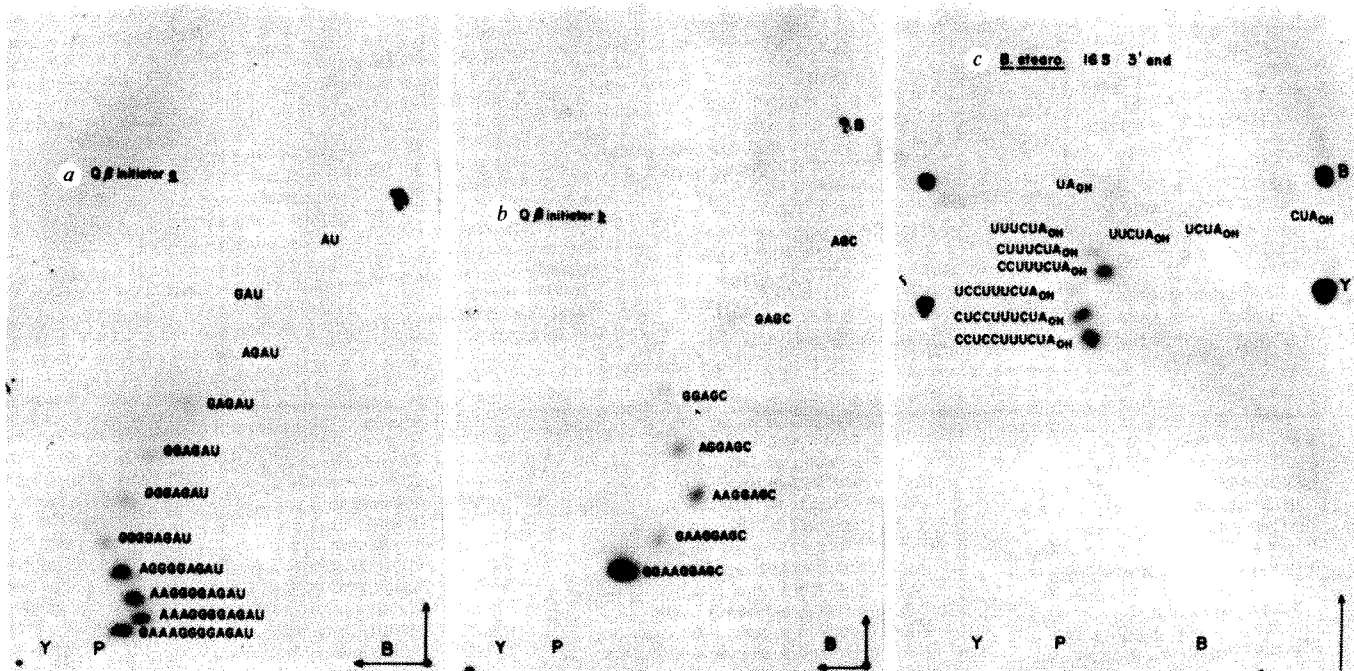


Fig. 1 Partial spleen phosphodiesterase digestion products of Q β non-initiator fragments (a and b), and of the 3' terminus of *B. stearotherophilus* 16S rRNA (c). ^{32}P -labelled Q β RNA was prepared as previously described²⁷. *B. stearotherophilus* ^{32}P -labelled 16S RNA was isolated from late log phase cells grown at 65 °C in 50 ml of low-phosphate medium containing 4 mCi of $^{32}\text{PO}_4$ (New England Nuclear). The growth medium was that described in reference 27, but was buffered with 0.02 M bis-Tris (Sigma), pH 7.0, and was supplemented with 1/20 volume of R broth¹¹ and with trace elements¹¹. RNA was extracted with hot sodium dodecyl sulphate (SDS) and phenol after treatment of cells with lysozyme and EDTA²⁸. 16S RNA (specific activity c.p.m. $2 \times 10^5 \mu\text{g}^{-1}$) was isolated by sucrose density gradient centrifugation. The sequences of the Q β polypurine tracts were determined by partial digestion with the 5' exonuclease, spleen phosphodiesterase. The fragments were isolated from a total pancreatic ribonuclease digest of Q β RNA after fractionation in two dimensions: (1) electrophoresis on cellophel (Kalex) at pH 3.5; (2) homochromatography on DEAE thin layer plates (Analtech) with homomixture C diluted 1:1 with 8 M urea²⁰. To obtain reproducible digestion conditions for spleen phosphodiesterase, unlabelled RNA derived from the homomixture was removed from eluted oligonucleotides by chromatography on DEAE thin layers (Macherey-Nagel) with 1.5 M pyridinium formate containing 7 M urea²⁰. After elution with 30% triethylamine carbonate, pH 10, (without carrier RNA), the radioactive oligonucleotides were digested with 10 μl of 0.17 mg ml⁻¹ spleen phosphodiesterase (highly purified preparation, a gift from E. G. Niles) in 0.1 M ammonium acetate, pH 5.7, 0.002 M EDTA, 0.05% Tween 80 in the presence of 30 μg unlabelled rRNA at 37 °C. Aliquots were removed at 0, 1, 2, and 3 h and immediately frozen. Digestion products in the pooled aliquots were fractionated by electrophoresis on cellophel at pH 3.5 in the first dimension (right to left in the figure), followed by homochromatography with 1:1 diluted homomixture C²⁰ on Analtech DEAE thin layer plates in the second (bottom to top in the figure). The sequences shown above were deduced from the first dimension mobility shifts produced by the loss of a single nucleotide, and were confirmed by further digestion of each oligonucleotide with T₁ ribonuclease. Sequence analysis of the *B. stearotherophilus* 16S 3' end used the terminal T₁ oligonucleotide eluted from dihydroxyboryl cellulose¹⁹. Taken together with the results of Shine and Dalgarno¹⁸ (underlined sequence), data shown in Table 1 establish the sequence GAUCAC (Py $\sim$) UCCUUUCUA_{OH}. Partial digestion with spleen phosphodiesterase of both the largest fragment produced by U₂ digestion [C(Py $\sim$) UCCUUUCUA_{OH}, shown in c] and the intact T₁ product (not shown) establish the sequence: GAUACCUC CUUUCUA_{OH}. Spleen phosphodiesterase digestion conditions were as described for the Q β fragments, except that 6 μg of carrier ribosomal RNA was used. The products were fractionated as described above for the Q β oligonucleotides, and were analysed by digestion with pancreatic ribonuclease (0.6 mg ml⁻¹, 2 h, 37 °C, in 0.01 M Tris-HCl, pH 7.5) after derivatisation with carbodiimide²⁰.

While initial work indicated that the *B. stearotherophilus* 16S terminal sequence^{3,18} was different from that of *E. coli*^{1,5-7}, our further analysis shows that the two sequences are identical except that the 3' terminal adenosine in *E. coli* rRNA is replaced by UCUA_{OH} in the thermophile. Thus, the rRNA pyrimidine tract which is complementary to mRNA initiator regions appears in both 16S molecules and cannot be the basis of the different mRNA recognition patterns shown by ribosomes from the two species at 49 °C.

Although contributions to messenger recognition by other regions of 16S RNA have not been ruled out by our results, variation in one or more of the protein components seems likely to be responsible for specificity differences between *E. coli* and *B. stearotherophilus* ribosomes. In fact, reconstitution studies¹¹ which measured the capacity of hybrid ribosomes from these two species to recognise the three R17 initiator regions showed the 30S protein fraction (rather than the 16S RNA) to be the primary specificity determinant. One contributor²² is apparently protein S12, which may exert its effect by altering the association of initiation factors with the ribosome (see ref. 23 for a discussion). In addition, Isono and Isono²⁴ have shown that protein S1 is either absent from or

functionally distinct in *B. stearotherophilus* compared with *E. coli* ribosomes; they further reported that addition of *E. coli* S1 to *B. stearotherophilus* ribosomes at 39 °C allows efficient translation of the phage f2 coat and replicase cistrons²⁵. We conclude that molecular models explaining functional differences between ribosomes should not be formulated on the basis of limited sequence information at the extreme 3' termini of 16S or 18S rRNAs; both proteins and additional rRNA sequences may contribute significantly to initiation specificity.

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Repeated helical pattern in apolipoprotein-A-I

THE binding of lipids into globules by the serum lipid-binding proteins depends on the unique structures of these proteins. Many have a high α helix content¹⁻⁴ which is enhanced by the binding of lipid, and their amino acid sequences⁵⁻⁸ suggest that the helices are often 'amphipathic' with a long hydrophobic face buried in the lipid surface^{9,10} and an exposed polar face which interacts specifically with the charged head groups of phospholipids such as lecithin. Apolipoprotein-A-I (or -Gln-I) is the larger of the two major human high density plasma lipoproteins¹¹⁻¹³, with 245 amino acids and a helix content³ of about 70% in the native complex. Physical measurements^{10,14-16} and a study of the sequence^{10,11} suggest that in several of its complexes reconstituted from phospholipids, the protein has a loosely folded structure of between 9 and 13 helices which float in the surface of a disk-shaped bilayer 5.5 nm thick and 11 nm in diameter^{17,18}, rather as logs float on water. With pure lecithin and A-I protein, each complex contains two protein and about 200 lipid molecules¹⁹. The pattern of non-polar groups in A-I protein is unusually regular, even for a helical structure, and this suggested that the sequence may have evolved by internal gene duplication²⁰ in the same way as some other long repetitive structures^{21,22}. Barker and Dayhoff²³, Fitch²⁴ and I independently found a regular 11- or 22-residue repeat pattern in it. Here I report an analysis of the repeats and discuss their structural significance.

To study the repeat pattern, the sequence was compared with itself by matching together all possible segments of a chosen length, using the comparison matrix method²⁵. There is evidently a strong pattern of repeats (Fig. 1) based on a 22-residue unit, which is itself made of two similar 11-residue halves. Initially two sets of calculations were done to explore the pattern; one with a long span of 29 amino acids to look for weak repeats extending over a long range; the other with a span of 11 to look for shorter features. These showed that the strongest repeats involve residues 120-185, where a pattern of 22 amino acids occurs three times over. Next, these three segments were aligned with one another and used as a basis for interpreting the rest of the pattern objectively. To look for the most consistent alignment a family comparison matrix was calculated, which used an average score between any part of the sequence and corresponding parts of the three aligned units (with span 11 and several sets of threshold values).

The results suggest that the protein divides into several regions: three regular repeats of 22 amino acids, residues 120-185; a fairly strong continuation of the pattern (186-240) which stops short of the C-terminal region (241-245); a strong pattern in residues 84-119, apart from one gap near Trp 112; an interrupted fragment of the main pattern (47-78); residues 1-47, which show no significant trace of the repeat, even though

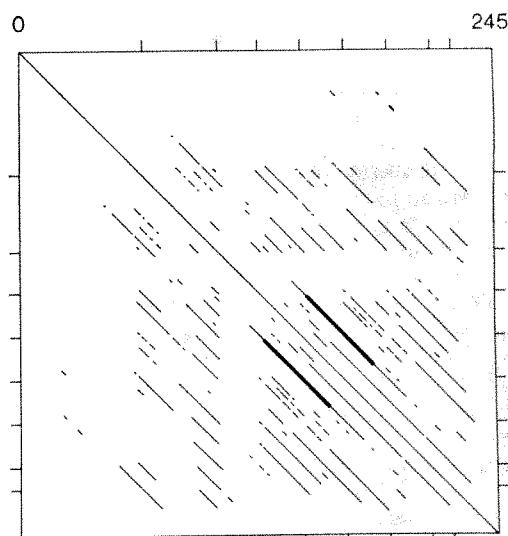


Fig. 1 Comparison matrix for human apolipoprotein-A-I with span 29 and all weights of 1. Points indicate centres of 1,226 off-diagonal spans with scores ≥ 125 . In a random sequence of this composition only 1 in 1,000 or 61 out of 5,970 spans should reach this score. Thick diagonals mark centres of 78 highly significant spans with scores over 146, for each of which the frequency predicted by chance is 1 in 10^6 . Marks along axes show proline residues at positions 65, 103, 124, 146, 168, 212 and 223.

the hydrophobic groups in residues 8-33 have the distribution characteristic of an amphipathic helix.

The best alignment (Fig. 2) is reasonably certain between residues 84 and 221, but the repeat of 11 residues within the basic unit of 22 leads to ambiguities elsewhere, so that several parts of the pattern might be misplaced by 11 residues (for example, near Pro 223). In spite of some uncertainty the observed pattern clearly fits the hypothesis that the protein contains a basic unit of 22 residues, repeated eight times in all.

How significant are the repeats? The answer depends on the null hypothesis, which is assumed. The simplest criterion is to compare the distribution of comparison scores with the expected one for a random sequence of the same composition (for a span length as long as 29, it is necessary to allow for the finite sequence length of 245 residues²⁶). Table 1 shows the distributions for the matrix with span 29 and weights of 1. The number of high scores is very significantly raised in the upper range above 110. In particular, the highest observed score of 162 has a predicted frequency of 10^{-9} and would be reached in less than one random sequence out of 10^4 . The expected top score of 138 is attained in 270 spans instead of two (note that the matrix is symmetric and so each span is counted twice). The odds against such a result in a random sequence are astronomical. Objection might be made that the

Table 1 Apolipoprotein-A-I comparison scores

Score compared with:	Expected frequency	Expected number	Found number
Self			
96	0.26	15,563	16,406
110	0.033	1,973	5,344
119	4.8×10^{-3}	287	2,228
125	1.0×10^{-3}	61	1,226
138	1.9×10^{-5}	1	270
155	2.6×10^{-8}	0	44
162	1.1×10^{-9}	0	2
Myoglobin			
93	0.25	9,555	10,541
105	0.046	1,730	2,386
112	0.011	428	625
119	2.1×10^{-3}	79	90
128	1.6×10^{-4}	6	2

Span 29 and weights of 1. Scoring scheme from ref. 25.

lipoprotein sequence is clearly not random, because of its high content of amphipathic helices, and that the repeats are a natural consequence of this structural feature.

To test this contention similar comparison matrices were run for apolipoprotein-A-I against other proteins with a high helix content: apolipoprotein-C-III (Ala)⁸, human serum albumin²³, and sperm whale myoglobin²⁷ (Table 1). In each case there was some enhancement of the number of high scores in the range 110–130, but no really high scores, above 133, were found. Thus the observed repeats in apolipoprotein-A-I are much stronger than can be accounted for merely by a high content of amphipathic helix. It therefore seems most likely that residues 47–240 have evolved by gene duplication from an ancestral unit of 22 amino acids which itself is a doublet of two sets of 11. The tests also suggest that apolipoproteins A-I and C-III do not have a common ancestor, as proposed by Barker and Dayhoff²³.

The characteristic structural features of the pattern (Fig. 3) can be seen in the five most regular repeats (99–207) which are derived from a sequence of the type: (1) Gln-Lys-Leu-Ala-Pro-Leu-Ser-Asp-Glu-Leu-Arg (11); (12)-Gln-Arg-Ala-Thr-Gln-His-Leu-Glu-Ala-Leu-Arg (22) (this is not an ancestral sequence but merely a selection of the most common residues in each position). The pattern of hydrophobic residues (period 22) forms a band along one face of the helix, whereas the pattern of polar groups, with a period of 11, consists of a triangle of three negative charges bordered by three positive charges which all lie on the opposite face. The charge distribution is peculiar to this protein and occurs very consistently in the repeats. It seems well adapted to interact with a dipolar lecithin head group in the way proposed by Segrest⁹.

The nature of the repeated motif strongly suggests that the evolutionary repeat unit of 22 residues is also a structural element, which builds an amphipathic α helix with a proline at the amino end, the surface charges of which form a pair of phospholipid binding sites spaced 1.7 nm apart. Notice that three turns of α helix use 10.8 residues, so that a repeat period of 11 or 22 residues is particularly favourable in a helical structure (compare the well known seven-residue repeat in tropomyosin²¹). One notable feature of the sequence as a whole is that an unbroken helix would form a continuous hydrophobic face in long runs including residues 8–33, 44–77 and 86–240. Thus the prolines may not form sharp bends in the lipid complex, but only cause small changes of direction in a series of colinear helices of a total length approaching 30 nm. Such

Fig. 2 Amino acid sequence (one-letter code) aligned to show repeat patterns of 22 and 11 residues. Circles pick out large non-polar groups. Note three strong repeats between 120–185, a gap after Trp 112, breaks at 79–83 and 46, and absence of regular pattern in residues 1–46 and 241–245.

1	(D E P P Q S P) D R (V) K D (L) A	15
16	T (V) Y (V) D (V) L K D S G R D (Y) V S Q (F) Q G S A	37
38	(L) G K Q (L) N (L) K (L)	46
47	(L) W D D V T S T (F) S K (L) R	59
60	Q E (L) G P (V) T E E (W) F N D (L) Q E K (L) N	78
79	(L E K E T)	83
84	G E (L) R Q E (M) S K D (L) E E (V) K	98
99	A K (V) Q P (Y) L D D (F) Q K (W) - Q E (M) E (L) Y R	119
120	Q K (V) E P (L) R A E (L) Q E G A R Q K (L) H E (L) Q	141
142	E K (L) S P (L) G E E (M) R D R A R A H (V) D A (L) R	163
164	T H (L) A P (Y) S D E (L) R Q R (L) A A R (L) E A (L) K	185
186	E (N) G A G R (L) A E (Y) H A K A T E H (L) S T (L) S	207
208	E K A K P A (L) E D (L) R Q G (L) L P V (L) E S (F) K	229
230	(V) S (F) L S A (L) E E (Y) T	240
241	(K (L) N T Q)	245

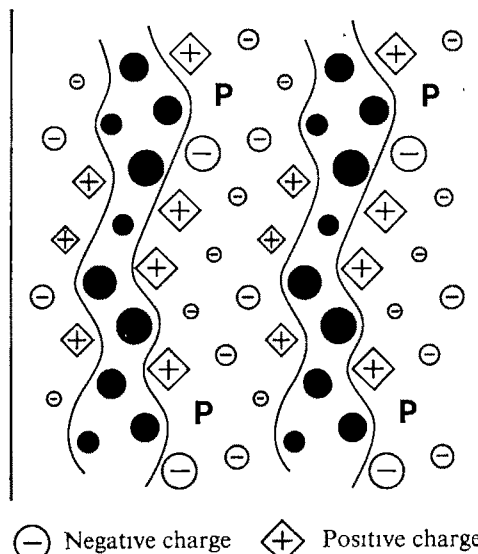


Fig. 3 Helix diagram of 22-residue repeat with 3.6 residues per turn. Sequence reads downwards right to left as if projected on to a sheet of paper wrapped twice round the helix axis and then unrolled. Each residue appears twice to show continuity of pattern clearly. P, Proline; ●, large hydrophobic group. Sizes are proportional to the numbers of each type in residues 99–207.

a structure could run round the circumference of the observed disk-shaped globule. On the other hand, the apoprotein itself without any lipid probably has a compact but loosely folded structure²⁸ which could form by stacking adjacent helices in anti-parallel pairs, rather as in the purple membrane protein²⁹.

Dr Fitch²⁴ has made an interesting phylogenetic analysis of the repeats and I thank him for correspondence.

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matters arising

Substitution of guanine for a specific base in tRNA by extracts of Ehrlich ascites tumour cells

WE previously reported the partial purification of an enzyme from Ehrlich ascites tumour that catalysed the incorporation of GMP from GTP into specific tRNA molecules¹. The active primer tRNAs for this reaction were found from *Escherichia coli*, Ehrlich ascites tumour, sarcoma 180, and tissues of mice infected with Friend virus, but not from tissues of normal mice. Among the ten purified tRNAs of *E. coli*, only tRNA_{II}^{Leu}, tRNA_{III}^{Ser}, tRNA_I^{Tyr} and tRNA_{II}^{Tyr} were active as primers. These results suggested that, in mouse, primer RNAs are specific to tumours or tissues infected with tumour viruses. We thought that guanosine triphosphate was the actual substrate, because the reaction was neither inhibited by creatine phosphate and creatine phosphokinase, nor by inorganic phosphate, but inhibited by inorganic pyrophosphate.

More recently, however, we have found that the incorporation of radioisotope from ³H-GTP into an acid insoluble fraction was not due to GTP but due to guanine contamination in the reaction mixture. It was shown by thin-layer chromatography on an Avicel SF cellulose plate that about 6% of radioactivity was accounted by ³H-guanine. We have found that α-³²P-GTP was not incorporated in this reaction, and ¹⁴C-GTP was not incorporated. It is very likely that ³H-labelled GTP apparently decomposed to yield ³H-guanine by self-decomposition due to radiation². In fact, the enzymatic incorporation of guanine showed an absolute requirement for acceptor tRNA (Table 1), but no energy source, for example ATP, was required.

Purified *E. coli* tRNAs were tested as acceptors for guanylation by the enzyme (Table 1). tRNA_{II}^{Tyr} and tRNA^{Asp} were very active. Slightly active tRNAs, tRNA^{Asn}, tRNA^{His}, tRNA_{III}^{Ser}, tRNA_{II}^{Leu} and tRNA_{II}^{Val}, were determined with the activities of charging of the specific amino acid. tRNA^{Asn} and tRNA^{His} were not contaminated with tRNA^{Asp} or tRNA^{Tyr}, but tRNA_{III}^{Ser}, tRNA_{II}^{Leu}, and tRNA_{II}^{Val} were found to be appreciably contaminated with tRNA^{Asp} or tRNA^{Tyr}. Thus probably only tRNA^{Tyr}, tRNA^{Asp}, tRNA^{Asn}, and tRNA^{His} act as acceptors. It can be concluded that the primer activity of

tRNA_{III}^{Ser} and tRNA_{II}^{Leu} in the preceding paper¹ is due to such contaminating tRNAs. Four tRNAs that were active in guanylation contained in common the modified base Q in the first position of anticodon^{3,4}.

Table 1. Incorporation of ¹⁴C-guanine into amino acid-specific *E. coli* tRNAs

<i>E. coli</i> tRNAs (A ₂₆₀)	¹⁴ C-Gua incorporated (pmol)	
tRNA _{II} ^{Tyr}	0.006	2.0
	0.05	4.3
tRNA ^{Asp}	0.05	4.7
	0.5	9.8
tRNA ^{Asn}	0.05	0.3
	0.5	1.0
tRNA ^{His}	0.05	0.5
	0.5	1.9
tRNA _{III} ^{Ser}	0.05	0.5
	0.5	1.7
tRNA _{II} ^{Leu}	0.05	0.2
tRNA _{II} ^{Val}	0.5	0.7
tRNA ^{Phe}	0.5	<0.1
tRNA ^{Glu}	0.5	<0.1
tRNA ^{Met}	0.5	<0.1
-RNA		0

The enzyme was partially purified by the same procedure described by Itoh *et al.*¹. The standard reaction mixture contained in a total volume of 0.25 ml, 22 μmol of Tris-HCl, pH 8.0, 3.2 μmol of MgCl₂, 0.02 μmol of 2-mercaptoethanol, 10 nCi of ¹⁴C-guanine (55 mCi mmol⁻¹), 30 μg of enzyme protein and each of the indicated tRNAs were added. After incubation at 36 °C for 20 min, the cold 5% trichloroacetic acid-insoluble materials were collected on membrane filters, and the radioactivity of the dried filters was counted by liquid scintillation counter. *E. coli* tRNAs were supplied by Dr S. Nishimura (National Cancer Research Institute).

After tRNA_{II}^{Tyr} labelled with ¹⁴C-guanine by the enzymatic reaction was isolated and treated with RNase T₂, it was fractionated by two dimensional cellulose thin layer chromatography. The radioactive product obtained by the treatment of ¹⁴C-guanine incorporated tRNA_{II}^{Tyr} with RNase T₁ was fractionated by two-dimensional PEI (polyethylenimine)-cellulose chromatography. The results obtained from these chromatograms and acceptor activities of tRNAs suggested that the enzyme catalysed the reaction to exchange the Q base for guanine at the first position of anticodon.

Farkas and his co-workers previously reported guanylation of tRNA in rabbit reticulocytes^{6,7}. They showed that the guanylated tRNA of rabbit reticulocytes were tRNA^{His} and tRNA^{Asn} (ref. 8). Okada *et al.* recently studied this guanylation reaction in order to identify the exact

position of guanylation and the guanylated species of tRNA. They showed that the Q base in the first position of the anticodon of *E. coli* tRNAs (tRNA^{Tyr}, tRNA^{Asp}, tRNA^{Asn}, tRNA^{His}) was replaced by guanine by the guanylation enzyme from rabbit reticulocytes⁹. It was found that tRNAs isolated from Ehrlich ascites tumour and AH7974 rat ascites hepatoma were active as acceptor for guanylation with the enzyme from Ehrlich ascites tumour, but normal mouse and rat liver tRNAs were not active (ref. 1 and unpublished data). Kasai *et al.* reported that the Q* (a derivative of Q) content of liver tRNA of AH7974 rat ascites hepatoma was twofold greater than that of normal rat¹⁰. One of the major factor of a difference of guanylation activity among these tRNAs is probably the modification of nucleoside such as Q and Q*. Furthermore, a specific structure or sequence common to these tRNAs may be recognised by the enzyme. It will be interesting to examine the structure of tRNAs from tumour cells concerning the guanylation activity.

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Amino acids in fossil woods

THE significance of the amino acid racemisation date for fossil wood from the Kalambo Falls Prehistoric Site, Zambia, recently reported by Lee, Bada and Peterson¹ requires clarification. The specimen used for ¹⁴C calibration (UCLA-1857), referred to throughout as 'Sangoan wood', is noted on Table 1 of ref. 1 as being 'Kalambo Falls, Site A, River Face (1959) Pit 3, No. 49 (9 ft 9 inch)'.

Because of apparent stratigraphic correlations when this specimen was excavated, it was entered in the field catalogue as being 'Sangoan' in context; this was the notation which was on the sample when it was supplied for racemisation dating. The stratigraphy of the Site A River Face was re-examined in 1963, however, and the section drawing², and field notes, show that this specimen came from a bed in the uppermost White Sands and Dark Clays, from which aggregates including diagnostic Acheulian artefacts were recovered nearby. (The 9 ft 9 inch refers to a field datum; the depth below the published trench datum is 13 ft 9 inch).

That the analyses indicate that the Site A specimen is younger than that from Site B is in agreement with the postulated stratigraphic relationships^{3,4} and palynological evidence⁵. Older ¹⁴C dates have been obtained on wood in equivalent, Site A contexts: >49,000 (GrN 3211); >52,000 (GrN 4228); 61,700 ± 1,300 (GrN 4896) = >60,000 (ref. 5). These would give an older racemisation date for the Site B wood.

Thus, both the analysed specimens from Kalambo Falls have Acheulian cultural associations, and the date of >110,000 for Site B has little bearing on the 'Acheulian-Sangoan transition.' It refers to the Moola Phase of the Bwalya Industry, not to the youngest Acheulian⁶. It is vital that specimens within the ¹⁴C dating range be obtained for calibration, in order to derive finite dates for both of these older woods.

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Ductile shear zones and basal aureoles in ophiolite complexes

ATKINSON¹ has recently attributed ductile shear zones in the Bay of Islands ophiolites of Western Newfoundland to the 'transportation and emplacement of oceanic crust and mantle on to a continental margin'. This may be so for some such zones, although the well-exposed basal thrust of the ophiolite-bearing White Hills slice in northern Newfoundland is not associated with either the ductile style of deformation or the 'low greenschist grade' assemblages that Atkinson describes. Moreover, in the Bay of Islands complex there are well defined ductile shears in both the interbanded transition zone between the harzburgites and the gabbros and in the lower parts of the gabbros themselves, that seem to be related to processes near accreting plate margins^{2,3}.

These are best seen in the Lewis Hills, where a wide variety of dunites, harzburgites and gabbros are intensely mylonitised (though rarely to greenschist facies) in zones from several metres wide down to a few centimetres. These zones, many of which are axial-planar to tight folds, are cut by much less deformed dykes and sheets of hornblende gabbro, which seem to have been derived from a tholeiitic parent. Other clear evidence of the overlapping in time of igneous and deformation events is provided by the presence of rotated inclusions of foliated gabbro in relatively massive wehrlite.

It seems then that the ophiolites were undergoing deformation in narrow zones of high strain before they were carried with the spreading ocean floor far from their place of origin at an oceanic ridge or possibly at a transform. It is unlikely however, that the basal aureoles formed in the same environment by the 'lateral flowage of oceanic crust and upper mantle' as Atkinson speculates. Most of the protoliths for these aureoles were distal continental

margin sediments and associated volcanics^{4,5} which were overridden by the ophiolites during their advance onto the continental margin.

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ATKINSON REPLIES—Berger and Jamieson's comments on 'ductile shear zones' in the Bay of Islands ophiolites refer to high-grade structures and assemblages related, in some way, to the formation of Late Cambrian lithosphere at an ocean ridge and to its subsequent lateral flowage during sea floor spreading. I am most familiar with these previously documented structures and agree with this interpretation of their origin.

Ocean floor spreading, however, does not emplace ophiolite sheets on to continental margins. My letter to *Nature*¹ dealt specifically with younger, lower-grade minor structures formed during a late Arenig-Llanvirnian period of ocean basin closure. Crustal shortening was accommodated by telescoping the North American continental margin along major thrust faults. These were singularly responsible for the transportation and emplacement of the Bay of Islands ophiolites on to the continental margin. Field observations of the faults show that they are sharp, clear-cutting surfaces—certainly in no way 'ductile' zones themselves. My reference to ductile deformation zones (DDZ) is in regard to minor structures found within the body of the Bay of Islands ophiolite sheet. These DDZ cut across and retrograde the gabbros, harzburgites, dunites, and 'aureole' rocks, as well as all those structures mentioned by Berger and Jamieson (including the mylonites of higher-grade DDZ).

Clearly, the observations of Berger and Jamieson and previous authors on relatively high-grade DDZ related to ridge and spreading processes are not being challenged here. The observations I have made concern a distinctly later event—one that was responsible for ophiolite transportation onto the continental margin some 40 Myr later.

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reviews

Dispelling fears over pesticides

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Pesticides and Human Welfare. Edited by D. L. Gunn and J. G. R. Stevens. Pp. 278. (Oxford University: Oxford and London, 1977.) Hardback £5; paperback £1.75.

THERE has been a great deal written and said about the alleged disadvantages and dangers of pesticides. This book has been produced in response to this publicity, and is sponsored by seven chemical companies from western Europe, including ICI and Shell from England. The book is intended for laymen and non-specialists, and so discusses, in a lengthy appendix, the meaning of some common technical terms, types of pesticides, their formulation and biological activity. The main text contains three parts, with a total of twenty chapters, each intended to be scientifically accurate, intelligible and authoritative.

There are four chapters in part one. The first three, on the world's food supply (W. R. Furtick), population growth (R. H. Gray), and vector-borne diseases (G. Davidson), introduce the problems that pesticides can help to solve. They combine to illustrate vividly the differences between the industrialised and the developing countries of the world. Data are provided for past and expected future increases in population and food production. It is estimated, for example, that pests consume about 30% of the potential food production in the developing countries, but they use only 10% of the world's pesticides, mainly for public health and exported crops. The developing countries have higher rates of population increase, and, just to maintain present standards, their food production will probably need to almost double during the next twenty-five years. The important point is made several times that methods of food production, medical services and birth rates cannot be considered in isolation, but are all components of a society's culture. Thus, the effective use of pesticides in food production implies a relatively sophisticated agricultural industry. The fourth chapter, by D. J. Ansell, explains some of the financial difficulties in increasing food production in the developing countries.

The second part, with ten chapters, provides examples of how pesticides improve crop yields, animal health and

food storage. The criterion by which pesticides are judged is their cost against the value of the increment in yield. G. Schumann suggests, particularly from German experience, that farmers get a return of between 1:3 and 1:9 for pesticides, going in exceptional cases as high as 1:100. Several other chapters comment, for specific crops, on the lack of precise figures: the introduction to the chapter on tropical cash crops discusses some of the reasons for this, and concludes that in general when pesticides are used their benefits are so obvious that detailed data are unnecessary. Such calculations, when possible, are based of course on a micro-economic analysis. D. E. Jacobs indicates that realistic cost-benefit analyses for an entire community need computer-aided models. J. P. Hudson touches on another aspect of the same problem when he explains that for commercial orchards, once the current standard spraying regimes have been used, it would be disastrous to stop spraying.

The final part, in six chapters, discusses complications of, and alternatives to, pesticides. It is clear that pest resistance to pesticides (J. R. Busvine) is a continuing major worry. The late J. M. Barnes presents a reassuring account of the hazards to people; the major risk comes from human carelessness, not from unexpected toxicity. I am not completely convinced that animals will always be adequate indicators of possible chronic toxic effects in man (p188), but that is a minor quibble. K. Mellanby discusses the "balance of nature" and makes the point that one can never prove that all hazards from pesticides are known. Nevertheless, he sees little cause for

alarm on this score, although I doubt whether we have too much evidence either way for some of the developing countries. D. P. Jones discusses the topical issue of energy costs and gains from pesticides. R. F. Glasser describes some of the controls imposed by governments on the development and use of pesticides, and suggests, as do several other authors, that the increasing costs of research are reducing the amount of research for new pesticides. It would have been interesting to have some comments on the fact that it is becoming more difficult to discover new potential pesticides at the primary screening stage. The final chapter, by D. L. Gunn, discusses alternatives to pesticides and concludes that pesticides will remain of major importance for the foreseeable future.

This book succeeds in its aims. It is clearly written, the few minor misprints causing no problems except for conventional (*sic*) on p212; most of the chapters read convincingly; and the book should inform and stimulate its intended audience. There are some omissions; for example, there are few if any data on quantities of pesticides manufactured and used, and few references to forestry or to herbicides in waterways. The major unanswered query was, where are we going? Clearly, in the short term, we will continue to need and use pesticides. In the long term, if pesticides merely enable us to keep one jump ahead of population increase, for how long will we be able to maintain that state of affairs? □

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Dynamics of atomic liquids

Atomic Dynamics in Liquids. By N. H. March and M. P. Tosi. Pp. 330. (Macmillan: London, 1976.) £25.

IT is difficult to decide for whom this book is intended. The level of discussion is high and the range covered is very wide. As may be expected the longest chapter is on charged fluids (plasmas, liquid metals and molten salts), a field to which the authors have

made important contributions. Apart from the conventional general discussion of the structure and dynamics of pure atomic liquids and binary liquid mixtures there are short specialised chapters on the helium liquids, the liquid surface and on critical phenomena. Few topics are discussed or explained in any detail, and a considerable expertise in theoretical physics is a prerequisite. Consequently this book cannot be recommended to the beginner. On the other hand it will not be very exciting for the expert, except as a reference book.

The material is not well organised and the

text does not read smoothly—quite apart from many errors of English and poor proof-reading. There is excessive direct quotation of formulae from specialised papers with inadequate indication of the physical assumptions involved. It is clearly difficult to decide in what detail to deal with this wide range of topics in a small book, but the authors have not found a happy solution. Many parts of the book, and its general arrangement, give the impression of very condensed notes for lecture courses. The lecture courses themselves were pro-

bably very good but they are not given here.

In view of the well established reputation of the authors and the significant contributions they have made to the theory of liquids it is to be regretted that they have not produced a book which does them justice.

This will be a useful reference work for experts and it is perhaps in anticipation of this that the price has been set very high.

J. G. Powles

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Remote sensing

Introduction to Environmental Remote Sensing. By E. C. Barrett and L. F. Curtis. Pp. ix + 336. (Chapman and Hall: London; Halsted/Wiley: New York, 1976.) £5.95.

THE authors' aim "has been to meet the needs of many students, administrators and scientists for an account of remote sensing which is essentially basic, explanatory and broad in its coverage", illustrating applications as well as techniques. There is certainly a need for such a book to be read, particularly by the administrators and scientists who control in various ways the support (or lack of support) and encouragement for the development of remote sensing and its applications. Does this book satisfy its declared aims?

A range of publications is now available on the subject of remote sensing, from popular accounts, through textbooks and books of collected papers to the *Manual of Remote Sensing* recently published (American Society of Photogrammetry: Falls Church, 1975) in two large volumes. The authors of the book now under review have re-written and edited some of these earlier accounts.

Just over half of the book is concerned with a "selective and illustrative" treatment of applications, covering a range from global climatology to urban studies, and this is perhaps the most useful half of the book for its stated purpose. The atmospheric aspects of the environment receive more attention in this book than in some others, which is not surprising in view of the authorship.

The first half of the book is concerned with the techniques rather than with the applications of remote sensing. The description of the physical basis of remote sensing and the radiation characteristics of natural phenomena reach about the intended level. The chapters on the techniques—that is, the sensors and data processing—are not so satisfactory. Too much previous knowledge is required for this to be included in a basic textbook, and an attempt has

been made to convey too much and too complex information in a small number of words. As one example, in nearly three pages on photographic processing the terms γ , MTF, film stability and granularity, chloride, chlorobromide and bromide papers, and sequential colour printing are introduced among many other topics.

Illustrations and references (including eight pages of colour figures), are both good and plentiful, except that quoted sources of illustrations are frequently not referenced.

It is unfortunate that the text contains enough misprints to be irritating. Occasionally these are factual: for example, the first introduction of ERTS 1 MSS imagery (Plate 3.3, p40) lists the spectral bands wrongly, although it must be said that they are frequently described correctly later in the book.

Overall, this reviewer gives a 'two cheers' reaction—good on applications and illustrations, and reasonably priced in these inflationary days, but barely suitable as a basic textbook on remote sensing techniques.

J. R. Hardy

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Human hormones in health and disease

Hormones in Human Blood: Detection and Assay. Edited by H. N. Antoniades. Pp. xvii + 810. (Harvard University: Cambridge, Massachusetts and London, 1976.) £37.10.

THE editor of this multi-author book, Dr H. N. Antoniades, quotes with approval in his Introduction the 1960 statement of Dr G. W. Thorn in his foreword to *Hormones in Human Plasma* that it would be impossible "ever again, to contain within one single volume all information pertaining to this important subject". The dust cover states that *Hormones in Human Blood* "will be an indispensable companion to laboratory workers engaged in clinical and research efforts to measure and understand the role

of human hormones in health and disease". With the book priced at £37.10 this claim merits close examination.

This volume contains 46 chapters contributed by 69 authors covering some 800 pages. The three initial chapters are concerned with plasma proteins and plasma fractionation techniques. The next eight chapters deal with basic aspects of radioimmunoassay methodology. The subsequent and largest section of the book (30 chapters) covers polypeptide hormones and the last section (five chapters), non-polypeptide hormones. A fairly rapid poll of the references quoted suggests that most chapters are completely up-to-date, with 1973 references and 1974 publications of the chapter authors; some go into 1975 and the chapter on growth hormone notably contains 1976 (in press) references which is remarkable as this is the publication year of the book. Does one congratulate the latter authors or identify them as the tardiest contributors?

If it is impossible to be complete in this area how far short of completeness does the book fall? Inevitably it must be in the most rapidly moving new areas rather than in new aspects of existing hormones. For example, of the gastrointestinal hormones only gastrin and glucagon-like substances are considered. This omission is perhaps made more disappointing by the imbalance of the material included. Thus the coverage is considerably affected by the editor's particular interest in protein fractionation and insulin. There are five chapters on insulin immunoassay and five chapters on insulin bioassay. In addition some of this information is also covered in the general chapters on radioimmunoassay methods. One feels, therefore, that a more satisfactory endocrine cover could have been achieved without expanding the book by pruning some of this dated material.

Nevertheless the breadth of coverage provided means that it is impossible for a single reviewer to give an authoritative general opinion of the quality of the contents. Most of the authors are very well established and actively working in their subjects, and therefore their contributions must be very valuable. Inevitably, in looking in detail at the areas with which one is most familiar, one finds defects. For example, must laboratory workers accept the dogmatic statement that the pre-precipitation double antibody method "produces a less sensitive assay than post-precipitation methods", for which the only justification cited is a previous undocumented assertion of the same nature?

One cannot help feeling that the modern laboratory worker in this rapidly moving area will prefer to consult the original literature for most of the methods but will be glad if somebody not too far away has found the money to buy the book.

C. N. Hales

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Super Proton Synchrotron project

Europe's Giant Accelerator: The Story of the CERN 400 GeV Super Proton Synchrotron. By Maurice Goldsmith and Edwin Shaw. Pp. 288. (Taylor and Francis: London, 1977.) £13.

In telling the tale of the Super Proton Synchrotron (SPS) project, Maurice Goldsmith and Edwin Shaw have produced a book that is both fascinating and frustrating. Although it is explicitly stated that the views and comments presented are those of the authors and not the official opinion of the European Organisation for Nuclear Research (CERN), the authors do not often stray from the party line, and the text tends to glorify CERN and praise the success of the SPS. And why not—it is a story to be proud of.

Beginning with a brief and rather high-minded justification for high energy accelerators the authors move straight into the political wranglings that continued for so many years before the SPS project was accepted and funded. A detailed description of the birth-throes is given but its conception is handled more discretely. It will be difficult for an uncommitted reader to become emotionally involved as the political and scientific justification for the SPS is inadequately presented. The reader needs to be emotionally involved to appreciate the detailed description of the implementation of the project which is presented in the rest of the book.

The discussion of the fight for acceptance of the SPS project is fascinating. The brevity of the discussion is frustrating. After rather full-some praise of the CERN convention—the rules by which CERN operates—it comes as a shock that it did not seem to work too well in the case of the SPS. More comment on this would have been welcome, as would be additional discussion of the roles of the various member states during the negotiations. One gets the impression that only a small part of the story has been told—enough to whet the appetite but insufficient to give full satisfaction.

Turning to the building of the machine itself, the authors describe the process in great detail in separate chapters dealing with individual aspects of the problem: surveying, magnets, power and water supplies, radio-frequency acceleration, and so on—topics beloved by engineers and belittled by everyone else, but presented here in an exciting style. Aspects often taken for granted are revealed as complex technical problems and their solution is surprisingly interesting, not

least because of the clear way in which they are presented.

Apt portraits are given of most of the key personnel in the project—a device which adds a great deal of interest to the technological descriptions but, even so, in places far too much technical detail is included and the story becomes difficult to follow. In general, however, there is a good blend of personal interest, technical detail, explanation and discussion of management techniques, which makes a very readable story.

Although the text is fascinating, the layout of the book is frustrating. Pictures are liberally sprinkled throughout; most are of interest but others are fatuous, and the text is fitted into the remaining space after huge margins have been accommodated. If a picture

is encountered, on turning a page one has to search frantically for the text; often there isn't any. Even when there are no pictures the text does not begin until nearly a quarter of the way down the page.

It would have been preferable to expand the scope of the book to put the SPS project into context and explain more fully the motivations and use to which it will be put. This could have been done without increase of the (already high) cost, at the expense of reducing the space taken up by pictures.

This book is one which can usefully be read even though it is produced as if it were a glossy handout for a publicity department. **Stuart Sharrock**

Stuart Sharrock is Physical Sciences Editor of Nature.

Organic reaction mechanisms

Reaction Mechanisms in Organic Chemistry. By Florin Badea. Pp. 701. (Abacus: Tunbridge Wells, Kent, UK, 1977.) £18.85.

THE investigation of organic reaction mechanisms by the application of kinetic measurements originated with Lapworth's work on the halogenation of acetone, at the beginning of the present century. It was not until much later, after 1930, and especially through Ingold's work on substitution reactions, that mechanistic studies became accepted as the cornerstone of the scientific basis of modern organic chemistry. Almost any recent textbook of organic chemistry, even at an elementary level, reflects this view. Several more specialised treatises, including the famous ones by Ingold and Hammett, have given more detailed analyses of the problems of reaction mechanisms in relation to the structure of organic compounds. The work now under review is a recent addition to the literature in the second category. Its author is Professor of Organic Chemistry at the Polytechnical Institute, Bucharest, Romania, and we learn that his research experience included a period in Donald Cram's laboratory at UCLA. The book is well translated (by the author, with help) into readable, though not altogether faultless, English.

The text starts with an introduction to group theory, an account of the Hückel molecular orbital (HMO) method, some rudimentary thermodynamics and statistical mechanics, a statement of the principles of chemical kinetics, and a brief survey of reaction intermediates. These sections are

followed by a detailed treatment of mechanisms of a large number of selected reactions, beginning with aliphatic substitution and ending with cycloadditions. The level of the treatment is intended for postgraduates and advanced undergraduates.

In its 700 crowded pages the book manages to include a vast amount of material and many references. Much of the information is of recent date, and it is not surprising that frequently the aptness of its selection is at least debatable. Clearly, in such an enormous undertaking, there are also bound to be errors of fact or interpretation. Some of these are excusable and could prove a challenge to a good student. The author does not generally attempt unconventional interpretations or to resolve conflicting views. He is not afraid to show his limitations and to leave the reader to develop his own critical faculty by sifting the arguments presented.

Probably the least satisfactory aspect of the book is the absence of any coherent attempt to explain the current methodology of the elucidation of reaction mechanisms. This is not a text from which a student could gain a satisfactory understanding of the application of kinetic methods and become aware of its pitfalls, for example. The treatment of topics such as the role of pre-equilibria, the stationary-state hypothesis, kinetic salt effects, or kinetic isotope effects is inadequate and sometimes seriously in error.

Nevertheless, the book is quite good in parts but, as better introductions to the subject are available in the English language, it is unlikely to be widely adopted.

V. Gold

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Dynamics of fish populations

Fish Population Dynamics. Edited by J. A. Gulland. Pp. xi + 372. (Wiley-Interscience: London and New York, 1977.) £13.50; \$25.

THIS book describes how the dynamics of fish populations can be analysed in terms of the factors affecting their rate of growth, mortality and reproduction, with particular emphasis on the effects of fishing. There are sixteen contributors to the book, including the editor, and many of them have a long-standing international reputation in this field, so much so, that the chapters by Ricker, Gulland, Cushing, Regier, Larkin and Allen, and Chapman are bound to guarantee the book the success it well deserves. It is intended primarily for those already having some interest in fishery science, and for graduates and final-year undergraduates in biology and related subjects. Its form of presentation and style will make it a very 'usable' reference for fishery science undergraduates.

The book has been arranged in three parts. In the first chapter, Ricker describes the historical development of the subject and includes the quantitative methods, of increasing reliability, for describing the direct effects of fishing on stocks of fish and for predicting the catches expected to result from changes in the intensity of fishing or in the sizes of fish caught.

The next section describes some of the particular lines of investigation and methods used in more detail. Because direct observation of fishing in the oceans is difficult and expensive if carried out by special research vessels, scientists have for long depended very much on information from the commercial fisheries; Williams describes the practical problems met in collecting this data. This is a particularly valuable chapter for undergraduates and recent graduates in making them aware of the problems facing the fisheries scientist in obtaining reliable data.

The main lines of analysis as used at present, are reviewed by Gulland, whereas further lines of advance are explored by Rothschild (concerning the concepts of fishing effort and fishing mortality), by Cushing (the problem of the relation between adult stocks and the number of young produced) and by Regier (the position of the fish stock in the whole ecosystem).

The third part of the book looks at how the ideas described earlier have worked out in practice when applied to actual populations. Fish population dynamics grew up in the North Atlantic and North Pacific and much of the work was done on three fish—cod, plaice and the five species of Pacific salmon. The present state of understanding of these species is reviewed by Garrod, Bannister and Larkin, respectively. Population studies and the application of these studies to practical problems, are now worldwide. Murphy looks

at the clupeoids, representatives of which are found in most parts of the world, and Rothschild and Suda look at the larger tuna species, most of which are found in all the warmer oceans. The studies reveal how many of the ideas developed in relations to a few fish in northern temperate areas have been usefully applied to very different areas and to very different fish, but Holden shows how some basic differences in the biology of elasmobranchs require some important changes in approach. Finally, Allen and Chapman examine some aspects of the dynamics of whale stocks and in so doing give us a very useful history of whaling.

For those with little understanding of statistics this is not a book to shy away from, as it is most readable and easily understood. It is also not without its lighter moments, as when Gulland (p70), when referring to the difficulty of making direct observations on fish at sea in mid-winter, states that it "involves a large, seaworthy ship costing hundreds of pounds to run. Most fishery research laboratories have one

or more specially equipped research vessels to carry out this sea work, but their high running costs and the need to justify their even higher capital costs often cause them to hang like gold-plated albatrosses round the necks of the directors of the laboratories concerned."

In addition, the political aspects of fisheries appear from time to time, as when Larkin (p183) refers to the fact that there is no international agreement that excludes participation of all-comers in the harvesting of salmon beyond narrow coastal limits and, as he says, "at present prices for salmon, it seems only a matter of time before someone decides to test the political wind."

This is a relatively expensive book but is one which the budding fisheries scientist should not be without. **Derek Mills**

Derek Mills is Lecturer in Ecology and Fisheries Management in the Department of Forestry and Natural Resources at the University of Edinburgh, UK.

Continental drift

Continents in Motion: The New Earth Debate. By Walter Sullivan. Pp. xiv + 399 + 29 plates. (Macmillan: London and Basingstoke, 1977; McGraw-Hill: New York, 1974.) £6.95.

THIS book is aimed at the general intellectual, non-specialist reader and is undoubtedly the best book on continental drift which has yet appeared for this type of market, although its numerous anecdotes and overview makes it interesting reading even for the specialist. It was originally published in the United States in 1974 and, although it is regrettable that it has not been up-dated, the information it contained at that time was so up-to-date that subsequent data have only extended and modified certain concepts. This new publication is therefore only slightly dated.

It is a large well produced book and contains 29 colour photographs, although some of these could have been dispensed with and further black-and-white diagrams inserted. It is written, as would be expected by the Science Editor of the *New York Times*, in the best journalistic tradition—with an easy style, full of human as well as scientific interest, yet avoiding the slickness and doomwatch aspects which often spoil such books. The drawback for a non-American reader is the emphasis on American findings: most examples are taken from America or American authors, although the British contributions are amply acknowledged. Nevertheless, Arthur Holmes

still only gets a few lines, whereas the Velikovsky farce receives almost a full chapter.

There are hardly any factual errors and most do not really matter for the audience at which it is aimed, although diagrams of the *expected* magnetic anomaly patterns in the western Mediterranean should not be portrayed as the actual pattern, even schematically.

I was more disappointed that Sullivan had not really contributed himself, although this is understandable as he has mainly been a bystander. Throughout, however, there was a sneaking feeling that he has already thought of, but not voiced, the questions which the specialists should be asking themselves, but which have not yet been recognised as the real problems.

The poorest coverage is on mechanisms as the concentration is very much on plumes, with brief consideration of the possibility of shallow convection, but virtually no regard to the probable dominant factor, large scale mantle-wide convection; but this may merely reflect the reviewer's prejudices! Surprisingly, the metal-ores, other than those of the seafloor, receive little attention, although petroleum and particularly geothermal energy resources are covered. But to ask for more is really a compliment to what has been included.

The book can be unreservedly recommended to the general reader, and even specialists would find £6.95 could provide them with numerous insights and amusement, even if they learn few new facts. **D. H. Tarling**

D. H. Tarling is Senior Lecturer in the Department of Geophysics and Planetary Physics at the University of Newcastle upon Tyne, UK.

newly on the market

These descriptions are prepared by the staff of *Nature* on the basis of material provided by manufacturers. The Reader Enquiry Card faces the inside front cover.

Plasma Emission Spectrometer. Technation. The Spectraspan IV, a single element plasma emission spectrometer can perform quantitative and qualitative analyses of trace concentrations including refractories and some non-metals. It has a high sensitivity even in the presence of complex matrix solutions with solids contents as high as 20%. The high intensity excitation source is a d.c. argon plasma which operates at low power but produces temperatures as high as 10,000 K. An integral microprocessor increases efficiency and allows routine performance checks.

Circle No. 42 on Reader Enquiry Card

Radiation dosimeter in SI units. Dosimeter. A radiation dosimeter in SI units, the Model 883 is calibrated 0–129 $\mu\text{Ci kg}^{-1}$ and 0–500 mR.

Circle No. 43 on Reader Enquiry Card

Graphite furnace. Perkin-Elmer. The HGA-2100 graphite furnace, a flameless sampling system is for use with atomic absorption spectrophotometers has the new gas stop and mini gas flow system which controls sensitivity during atomisation. This graphite furnace can determine most metallic elements with detection limits up to 1,000 times better than those obtained with conventional burner systems. Solution volumes as small as a few tenths of a microlitre can be analysed as easily as larger samples of up to 100 μl required when measuring extremely low concentrations or non-homogeneous materials. Addition of a temperature ramp accessory provides a convenient means of automatically increasing temperature during all three phases of operation, dry, char and atomise. The HGA-2100 can also analyse many solid samples directly—materials that have been successfully analysed without prior dissolution include plant tissue, polymers, finger nails and paper products. The determination of metallic elements in difficult matrices is also simplified by the design of the gas flow and sample introduction systems. Lead can easily be determined at low levels in 6 N solution of sulphuric acid.

Circle No. 44 on Reader Enquiry Card

UV-Vis spectrophotometer. Perkin-Elmer. The dual wavelength, double beam, UV-Vis Spectrophotometer, Model 556, is suited for applications involving turbid samples, mixture analysis and other biochemical and industrial applications. Two separate diffraction grating monochromators, with independent selection of wavelength, produce the sample and reference beams which pass along identical paths through the sample cell. To record the spectrum of a turbid sample where no reference solution exists, one monochromator can be fixed and the other set to scan. Alternatively, the two monochromators can be fixed a few nanometers apart and both scanned together to record the first derivative of a spectrum. Double beam operation is with both sample and reference beams taken from one monochromator. Regular difference spectra can be recorded with a high performance level.

Circle No. 45 on Reader Enquiry Card



Philips new PW 1720 table-top X-ray generator equipped with Debye-Scherrer, Universal flat plate and Guinier cameras.

Table-top generator. Philips. The PW 1720 top measures 750×700 mm and with standard Philips tube shield, will easily accommodate single crystal goniometers and Debye-Scherrer, Guinier or Universal flat plate cameras. A vertical goniometer for diffractometry can also be fitted. The height of the generator top is 380 mm, enabling it to be placed on a table and provide an ideal working height for camera alignment. The basic generator is full wave rectified, with rated power consumption of 2,500 VA max., and has full tube current stabilisation, continuously adjustable between 5 and 60 mA. Current stability is <0.3% per % mains voltage variation of $\pm 10\%$, with drift <0.2% per $^{\circ}\text{C}$ at ambient temperature.

Circle No. 46 on Reader Enquiry Card

Thermal imaging camera. Marconi-Elliott. The pyro-electric camera has a special Vidicon camera tube which responds only to heat radiated from a subject with no contribution whatever from light in the visible spectrum. The camera gives an output which is completely compatible with standard 625 line TV equipment. This, and its portability, has been demonstrated by its use as a 'roving' camera. Hitherto thermal imaging cameras involved highly complex and expensive forward looking infrared (FLIR) systems, based on the use of a large matrix of discrete heat sensing elements, in conjunction with a very high speed mechanical scanning system. Their cost greatly limited the application of thermal imaging techniques. The Vidicon camera, whose construction and operation is similar in principle to that of a conventional TV camera, can extend the technique to a variety of industrial, medical and even domestic uses. Working with virtually any TV monitor the camera can show pictures which bear a remarkable resemblance to those taken by cameras using visible light using only the pattern of thermal energy produced by the object. Thus 'hot spots' in rotating machinery, including for example, vehicle tyres, can be readily located as can thermal stress in power cables and soil patterns in aerial surveys. For medicine a camera of this kind could have application in thermography for the diagnosis of tumours. For surveillance, an intruder can be detected even in total darkness. Its use in defence equipment is also significant since, by not requiring to irradiate the object observed with infrared energy, the presence of the observer cannot be detected.

Circle No. 47 on Reader Enquiry Card

Programmable multiple reagent dispenser. Dynatech. The Dynadrop delivers up to 12 individual reagents to a Microtiter plate in quantities from 10 to 200 μl within an overall accuracy of $\pm 2\%$. Individual reagents are contained in a rack of test tubes which in turn is located in a low pressure reservoir. An integral pump provides filtered supply air at up to a maximum of 5 lbs inch^{-2} . The reservoir, connecting tubes and dispense manifold are all autoclavable.

Circle No. 48 on Reader Enquiry Card

Digital radiometer. Ultra-Violet Products. The J-260 digital radiometer measures UV intensities rapidly and automatically. Digital readout in radiometric units is within a broad dynamic range of $1 \mu\text{W cm}^{-2}$ to 199.9 mW cm^{-2} with accuracy, traceable to NBS, $\pm 1.0\%$ of full scale. High sensitivity for measuring low level irradiance is to the order of ± 1 digit. The radiometer is available with cosine-corrected sensors for measuring at 254, 297 or 365 nm. It is fully portable, uses alkaline or rechargeable NiCd batteries and is supplied complete with choice of sensor, batteries and recharger.

Circle No. 49 on Reader Enquiry Card

Navigational echosounder. Marconi. The Seahorse features possibly the largest chart to be found on any known navigational echosounder, an integral readout, automatic range change facilities and a depth alert. Optional extras are a large size remote digital readout and a large analogue meter-type display. Seahorse provides two depth ranges 0–100 m and 0–1,000 m, and a depth alert system.

Circle No. 50 on Reader Enquiry Card

Portable infrared gas analyser. Wilks Scientific. The Miran 104 portable gas analyser is a light-weight 14.5 m variable path length gas cell which, when allied to the Miran spectrometer, produces a high sensitivity and specificity analyser.

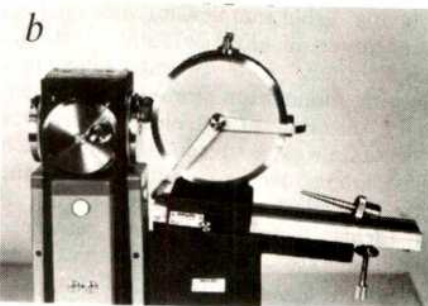
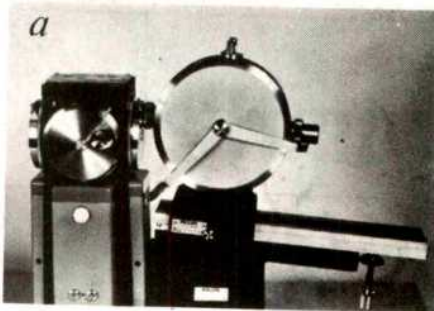
Circle No. 51 on Reader Enquiry Card



Wang PCS II

Deskette computer. Wang. The Wang PCS II Personal Computing System, is the first desktop computer available with minidiskette storage devices. The PCS II has a 1,024-character CRT screen; a typewriter-like keyboard with separate numeric keypad and 32 special function keys for data entry and control; a high-speed processor with two memories: 8 kbytes of user-available memory and 42.5 kbytes reserved for the 2200 operating system and BASIC language interpreter, and either one or two minidiskettes, each of which stores 89,600 bytes of information. The complete PCS II system weighs 29 kg.

Circle No. 52 on Reader Enquiry Card



a, Debye-Scherrer cameras. Camera correctly installed—safety arm activates microswitch to open beam shutter.
b, Part of camera (exit tube) removed—safety arm de-activates microswitch to close beam shutter.

Debye-Scherrer Cameras. Philips. The PW 1024/30 and PW 1026/30 cameras can be used with any Philips tube tower provided with an external safety microswitch, and use the standard diameters of 114.83 mm and 57.54 mm respectively. An important safety feature is a counter-balanced arm which can only activate the safety microswitch—to open the tube tower shutter—when the camera is correctly installed. If the camera, or any part of it, is removed the arm releases the microswitch and the tube tower shutter immediately blanks-off the X-ray beam. The arm also prevents the inadvertent use of an entry collimator in place of the correct exit tube. Exit tubes are now of one-piece construction so that removal of the lead safety glass is impossible.

The camera body and pedestal are formed as a single integrated unit so that alignment relative to the tube tower exit is automatically correct. Accuracy is improved because the collimator and exit tube are always in correct alignment.

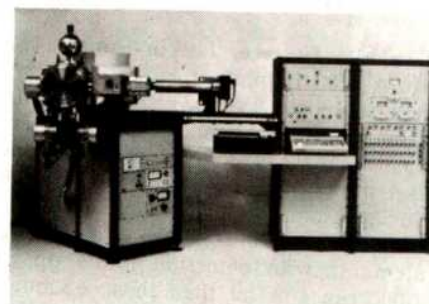
Circle No. 53 on Reader Enquiry Card

Portable noise monitor. Radiatron Instruments. A self-contained portable noise monitor, designed to calculate and print-out community noise levels the Model 614 will print out a wide variety of noise data. The instrument can be equipped to provide a threshold-controller single event printout (such as a flyover) including start and stop time and maximum noise level.

Circle No. 54 on Reader Enquiry Card

Mass spectrometer. Varian MAT. The MAT 260 spectrometer system is intended for automatic precision analysis of isotope ratios in solids (U, Pu, Rb, Sr, Pb and others). A programmable desk top calculator HP 9825 A is an integral component of the mass spectrometer system, optimising the ion source, controlling analysis procedures as well as evaluating and listing results. Twelve masses can be preselected for each measuring cycle, the user having the choice of manual 'one-off' measurements and fully automatic analyses of up to 13 samples mounted in a sample magazine. The exceptional performance of the MAT 260 is due to the high efficiency of a totally new ion source employing two einzel lenses, and the near 100% transmission of the newly developed stigmatic focusing analyser, the resolving power of which corresponds to that of a conventional 46 cm radius system. System sensitivity is better than one ion per 1,000 atoms of uranium. Internal reproducibility is better than 7×10^{-5} . Optimum vacuum conditions are generated by a turbo-molecular pump, two getter ion pumps and a cryo pump. The pumping system is almost maintenance-free and requires no cooling water connection. Devices for sample handling and preparation are included in the system.

Circle No. 55 on Reader Enquiry Card



Varian MAT 260 Spectrometer system

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Circle No. 56 on Reader Enquiry Card

nature

9 June 1977

Recombinant DNA: local or federal regulation?

AFTER raging through college campuses and state and local government offices throughout the United States, the debate over the risks and benefits associated with recombinant DNA experiments has now reached a critical juncture in the US Congress. In the next few weeks, committees in both the House of Representatives and the Senate are expected to approve their own versions of legislation to regulate recombinant DNA experiments. Although substantial differences of opinion between the House and Senate committees are emerging on key aspects of the legislation, one point in particular has become the focus of a major lobbying effort by members of the scientific community: the extent to which federal regulations governing recombinant DNA activities should over-ride controls adopted by state and local governments.

Some important points of principle are at stake here, and it is no surprise that the matter has become the centre of a controversy. The dilemma facing Congress is this. On the one hand, it would make no sense to have a patchwork of state and local controls on recombinant DNA research so that experiments were regulated more tightly in one city than in another. Genetically modified micro-organisms, after all, would not respect political boundaries, and a uniform set of national (or, preferably, international) controls would therefore make scientific sense. But on the other hand, the debates which have resulted in state and local governments adopting their own regulations have witnessed considerable public participation in scientific decision-making and the forging of new links between universities and their surrounding communities—ideals often given lip service but seldom put into practice. If the federal government takes away the right of local governments to set their own recombinant DNA regulations, it would risk stifling such developments. Moreover, there is some concern about the political implications of usurping a community's right to decide whether potentially hazardous activities should be permitted in its midst.

Needless to say, the scientific community has come down heavily on the side of federal pre-emption. Indeed, some scientists have publicly stated that they would support federal legislation only if it establishes uniform national regulations. The most prominent statement of such a position came late in April, when the National Academy of Sciences approved a resolution urging Congress to write a provision into the recombinant DNA bill specifically stating that federal controls would over-ride all local regulations. The Academy's resolution, it is

worth noting, was endorsed by Robert L. Sinsheimer, of Caltech, a leading critic of current guidelines governing recombinant DNA experiments.

But there has also been weighty testimony from a few scientists and from state and local officials, who have argued that the intervention of local governments in the recombinant DNA debate so far has been beneficial and there is no reason to expect that arbitrary or excessively strict local control will be adopted in the future. Every state and local government which has acted so far has endorsed the NIH guidelines, added a few minor additional precautions, and adopted procedures to enforce them. Even in Cambridge, where town-gown relationships had been notoriously bad, the city council approved recommendations, put forward by a citizens' committee, allowing recombinant DNA experiments to go ahead at Harvard and MIT essentially under the NIH guidelines. The report of the citizens' committee, in fact, provides an excellent example of how a group of lay people can participate effectively in scientific decision-making.

Is it therefore possible to establish uniform national controls without shutting off legitimate and healthy local inquiries? Possibly. Although some key legislators are hanging firmly to the notion that states should be given free licence to do as they please, a consensus seems to be developing in support of a scheme which would allow local changes to national regulations only under special circumstances. Perhaps more important, the scheme would provide local communities with an opportunity to have some input into national regulations.

It would work like this. The Secretary of Health, Education and Welfare (or whoever administers the national regulations) would have to approve local changes to the national rules. Approval would be given only if the local regulations are more strict than the national rules and if the local government can demonstrate that there are special local circumstances or that they are necessary to protect public health. Opportunity would be given for public hearings on the proposed changes. In practice, if a local government can make a good case, it would be likely to trigger changes in the national rules.

Although the arrangements would limit the powers of local government in the recombinant DNA debate, it would give them more authority than they have in areas such as radiation standards and the regulation of food additives. In short it represents a reasonable compromise between total federal pre-emption and the possibility of widely varying local controls. □

Under a cloud of committees

From Sydney, Peter Pockley updates some recent developments in Australian science policy

THE science-and-politics scene in Australia in the first half of 1977 has been remarkable only for much running on the spot with accompanying huff and puff. The symptoms are characteristic of SIC, the Syndrome of Inquiry and Committee, which, whether caught adventitiously or implanted deliberately, is manifest through a distinct brake on progress.

Those looking for a plot behind every move of the present conservative government might adduce SIC as evidence of a concerted move to downgrade the national science effort, the end result being disastrous attrition of the research dollar through galloping inflation before any substantive action occurs. But, on unravelling who is doing (or not doing) what among the dozen or so federal government departments with a major stake in science and technology, it is not surprising to find little more than a classic case of uncoordination.

One positive aspect of SIC is the appearance it gives of greater sympathy within the Liberal/Country Party government towards science, notably its applied aspects, than obtained within its Labor predecessor, in spite of the fact that under Labor science policy discussions were more public than before. After 18 months in office, though, the Fraser government's interest is still expressed only by SIC and the maintenance, for the time being, of the status quo. Everyone now expects the funds for science to remain very tight while high inflation (11–15%, depending on time-scale and assumptions) and high unemployment (5.5%, the highest since the Depression years) dominate the economic climate. Nonetheless, there are some government inquiries and committees which have the potential, at least, to affect constructively the course of events.

Breathing space

Late last year, a three-man Committee of Inquiry was established to look thoroughly into Australia's research colossus, the Commonwealth Scientific and Industrial Research Organisation (CSIRO). The full-time Chairman, seconded for the purpose from the Australian National University, is Professor Arthur Birch, an organic chemist noted for his contribution to the contraceptive pill. The other two members of the committee are a financier and an industrialist. Their appointments caused some tremors that the

government was intending, through the inquiry, to commercialise CSIRO by demanding short-term financial benefits from its research.

The final clause of the comprehensive terms of reference for the inquiry required the committee to "examine, report, and make recommendations on the extent to which and the means by which programs of the Organisation could attract revenue both to support the conduct of ongoing or intended research and also in return for results achieved in research".

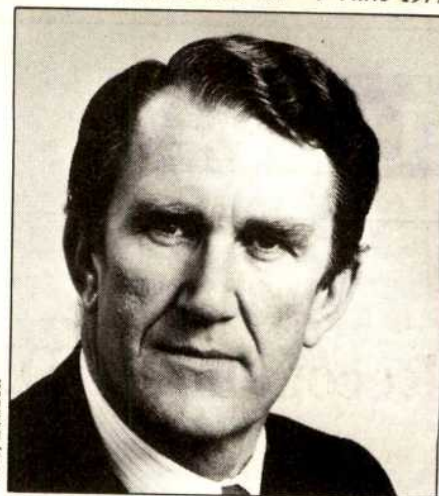
The inquiry, though, ranges far wider than this. While it was not intended as a mere cost-benefit study of the operations of CSIRO's 37 divisions and 100-plus operations and \$140 million-plus budget, the pressure was on the members to come up with an interim report early in the year. The urgency was felt because of the imminence of the retirement in March of CSIRO's Chairman, Sir Robert Price (formerly Dr Jerry R. Price). The inquiry was asked to answer the question of what kind of person is needed to head the organisation, but an organisation that before long would presumably have some new terms of reference from the government as a result of the inquiry's own recommendations, which would have been either tentative or unframed at that time.

The government solved the inquiry's dilemma by appointing, for 12 months only, a new chairman in Mr Victor Burgmann, a long-standing member of CSIRO's Executive. The inquiry now has some breathing space for its major tasks, on which it was originally expected to report to the Prime Minister by the end of July.

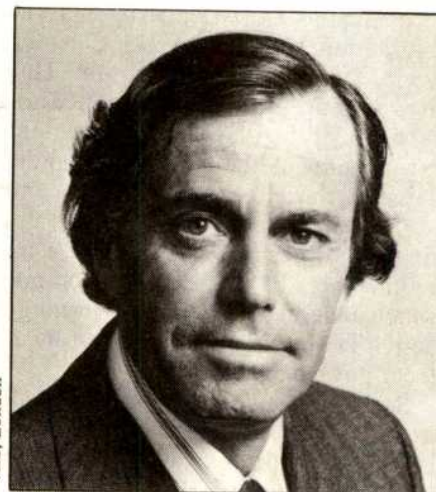
Professor Birch says the inquiry is "not trying to put a few band-aids on CSIRO, but is taking a hard look at the whole set-up—where it fits in between universities and industry, and between Commonwealth and State departments and instrumentalities. While the benefits from cost-benefit analysis of CSIRO's research will be very hard to measure, we are making strenuous efforts to gain a good idea of them."

The Prime Minister showed his close interest in the inquiry by issuing the commission himself and, although CSIRO is responsible under its Act to the Minister for Science, Senator James Webster, the report will go to Mr Fraser.

Professor Birch says he has no political connections and he hopes the in-



Malcolm Fraser, Prime Minister



James Webster, Minister for Science

quiry's recommendations ("not gospel from heaven") will be acceptable to both sides of Parliament, for he sees CSIRO's operations as "lying outside the range of normal party politics". Nevertheless, given the continuing silence of the Labor Party on matters scientific, it is likely that Mr Fraser will be able to remould CSIRO's terms of reference to his own liking.

Going autonomous

The longest running stage show in Australian science—the on-again, off-again saga of a science council in various costumes—has at last been concluded with an announcement that the independence, permanence and stability of a statutory authority would be granted to ASTEC, the Australian Science and Technology Council. ASTEC proper, however, still has to await the presentation and passage of legislation through Parliament.

At least ten years of lobbying of successive Prime Ministers and Ministers for Science finally bore fruit when Mr Fraser announced on 19 April that he had accepted, in large measure, the recommendation of the Interim

(continued on page 479)

Uranium uncertainty

THOSE on either side of the nuclear power debate who were hoping for a clear 'stop' or 'go' for uranium mining in Australia and uranium export have been left disappointed by the second report of the Ranger Uranium Inquiry. The report, prepared by Mr Justice Fox of the Australian Capital Territory Supreme Court and his two fellow commissioners, was published on 25 May by the Australian Government Publishing Service.

It included several surprises for the speculators, as indicated by the wild fluctuations in the share market for uranium mines immediately after the report's much publicised release. On the specific problems of mining uranium in the Northern Territory, the report, like its predecessor handed down in October 1976 which dealt more with the global issues of safeguards and controls, has left the ball firmly in the government's court.

- There is something in it for the uranium lobby. The Ranger mine is given a cautious go-ahead, though under much stricter and very specific (hence more expensive) conditions regarding environmental control.

- There is something in it for the environmentalists. It is recommended that "as proposed, and in the land setting which was proposed, the Ranger project not be allowed to proceed". Further and larger mines nearby in the uranium-rich Alligator Rivers province, the development of which was also halted pending the Ranger report, are recommended

"not to proceed, at least for the time being". The main mines delayed by the proposed sequential development are the Noranda and Jabiluka mines.

- There is even more in it for the aborigines whose claims to land in the area cover most of the uranium province. The report is strongest of all on this matter, recommending a trebling of the Kakadu National Park to cover the entire Alligator Rivers region, yet warning that the greatest threat to the environment and particularly to the welfare, well-being and culture of the Aboriginal people, might prove to be the large white population which the mining ventures might bring.

The Prime Minister, Mr Fraser, left for overseas on the day after the report's release. He has signalled that his discussions with President Carter and other leaders have uranium policy high on the agendas, especially since Mr Carter's staff have been saying, somewhat paradoxically for the layman, that the new US policy for containment of nuclear proliferation relies heavily on the export of Australian uranium.

While the Australian government has made no formal decision and has announced that a full debate will not occur in Parliament until the Budget session in August, few people doubt that mining will be allowed to proceed. The only real question is which mines will start and when. Meanwhile, the public relations and political lobbying of the pro- and anti-nuclear

forces will intensify, each side claiming to summarise accurately in a few words that the highly complex and voluminous Fox report supports their side.

On a wider front, however, Australia has joined the political bandwagon that is energy more by reason of following the leader (that is the USA and Europe) than of any urgency felt imperative locally. Australia is blessed with vast black and brown coal deposits, there are some modest oil wells and reasonable gas fields, and there has been a government policy of keeping down the prices of petroleum products. Despite the need to import increasing quantities of crude oil, few Australians have felt the effect of a genuine scarcity of energy. Politically, energy has not yet equated with votes.

Nonetheless, the Deputy Prime Minister, Mr Doug Anthony, has seized on the energy question as his rightful responsibility under his role as Minister for National Resources. His views on the matter are now accorded respectful space in the press, a good start to making energy a real issue. But, while there is much talk of "moving towards an energy policy", nobody is really saying what it is likely to be.

Mr Anthony has shown willingness to listen to expert opinion, and has set up an 18-person National Energy Advisory Committee chaired by top metallurgist, Dr Howard Worner. It is charged with advising on Australia's energy reserves, supply and demand, future costs, economy of use, research and technology.

In a welcome move, Mr Anthony has decided to release the Committee's advice for public information and debate. The first advice, released in May, commented with fulsome praise on President Carter's message to Congress on energy. It said that, despite obvious differences arising from the levels of consumption, "the basic conservation principles expressed in President Carter's statement are, in the Committee's view, directly applicable to the Australian situation". It advocates, for Australia, smaller cars, more effective use of public transportation, reduction of wastage, use of surface rather than air transport, rail rather than road transport where appropriate, and lowering speed limits on open roads. As with the Fox report, the government has given no indication of action to follow this advice.

Peter Pockley



Mary Kathleen uranium mine, circa 1961

Astronomy's own cloud

Meteorology and astronomy, two areas which seem to boil over periodically and publicly, have been placed under inquiry by the Australian Department of Science. Both committees of inquiry have been under way since late last year and were charged with reporting to the Minister for Science, Senator James Webster.

Neither report has been completed nor been made public. Some anxiety on this point is evident. The Secretary of the Department of Science, Sir Hugh Ennor, has only a few months left before retirement and, being the forceful character that he is, he doubtless wants to wrap up his work with the weather and star men safely settled for a while.

Relations between the Australian Bureau of Meteorology, headquartered in Melbourne, and the Canberra-based Department of Science have been tense ever since the bureau was transferred to the Department's responsibility under the Labor government. The committee was set up following regular and damaging leaks in the press of alleged confrontations between the bureau and the department. Its terms of reference included "making recommendations on the Head Office and Regional Organisational structure", but other terms cover all the bureau's functions.

The bureau has operated under its present act, the Meteorology Act, since 1955. The accompanying degree of independence was important to its Director, Dr Bill Gibbs, and to many users in this vast land mass where the rural industry and aviation services are peculiarly dependent on sound and comprehensive weather advice. The inquiry was seen by many as being the only method of resolving the bureaucratic conflicts. Significantly, the three-man committee did not include any professional meteorologist.

Things are a little different in astronomy. Ever since the early 1960s when Australia leapt into the big league of astronomical nations with the building of the giant 64-metre radio dish at Parkes, New South Wales, a clamour for large facilities has been maintained, and to a reasonable extent satisfied. From the astronomer's point of view, their requests made good sense. Despite its relatively low terrain, Australia provided the only geologically and politically stable platform in the southern hemisphere for observing stars obscured to northern telescopes.

To a certain extent the argument of uniqueness is no longer as strong

following the construction of some large telescopes in the South American Andes and Hawaii—at which stage other arguments take over, about maintaining a world lead, keeping the best scientists at home, and the prestigious PR that a nation gets from big telescopes. To back these arguments, the Australians now have a magnificently timed and very visible example of how astronomical research can lead to necessary (and profitable) application on Earth. At the end of March the Australian-developed 'Interscan' system for aircraft landing control in three dimensions was officially adopted as the next international standard. Interscan beat all comers from the USA, the USSR, Britain and Europe and is now the basis of a burgeoning industry. The microwave system was developed by the radioastronomers of the CSIRO Division of Radiophysics.

The Parkes facility was expanded in the mid-1960s to become an interferometer, and the main dish has recently been refigured at its centre to accept millimetre wavelengths. Among the other facilities around the country, the University of Sydney built its own radio interferometer—the so-called 'Mills Cross', named after its designer, Professor Bernard Mills—and an optical interferometer, Professor Robert Hanbury Brown's Stellar interferometer at Narrabri in northern New South Wales (now at the end of its useful life). CSIRO's Division of Radiophysics opened a magnificent solar observatory at Culgora, also in northern NSW, with Dr Paul Wild's Radioheliograph, together with optical solar telescopes of another division. Meanwhile, the Australian National University was expanding its optical observatory at Siding Spring in northern NSW, and in 1974 the joint Anglo-Australian Telescope Board opened its 3.9-metre telescope on the same mountain, then the largest optical telescope in the southern hemisphere. Beside it, the British also now operate a 1.9-metre Schmidt sky camera.

Enough for a nation of nearly 14 million people and few astronomers? Not so, said the astronomers, and asked for more—here remains our one chance of maintaining across-the-board, world-class excellence in a branch of science. Because of the sums involved for new facilities of significance, leading astronomers the world over have had to become politicians, and the Australians are no dullards at this game. Every minister responsible for science in Australia

has eventually cried for mercy—continuance of the ad hoc lump-sum handouts of earlier days could no longer be justified, they said, without the unanimous recommendation of an expert committee.

And so began an unceasing stream of references from ministers to committees which either never reached unanimity or had to be replaced because of political change. The latest act has been the establishment of yet another committee to end all committees, this one by Senator James Webster.

The committee, it was announced, was to be "an interdepartmental committee (IDC) chaired by the Department of Science. The Committee comprises representatives of the Public Service Board and the Departments of Education, Science and the Treasury". The IDC (of non-astronomers, it will be noted) was charged with inquiring into the range of astronomical research and facilities.

The public servants of the IDC would clearly be responsible for making the recommendations to the minister. However, an "expert sub-committee" was named to assist the IDC. And there was the rub, as far as the scientific community was concerned. For, while the names of the sub-committee were publicised (it is chaired by Professor Kevin Westfold of Monash University), the IDC remained anonymous in the official announcement.

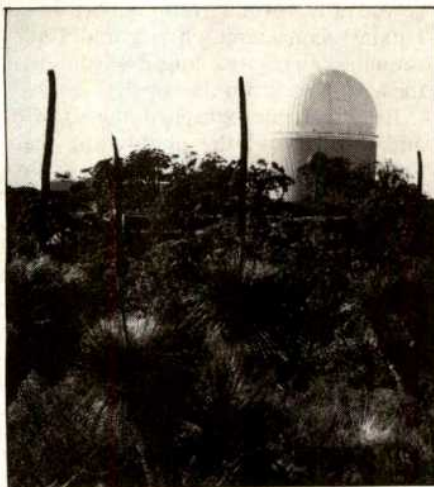
The gentle inquirer, however, would soon be told that the IDC was chaired by the redoubtable Sir Hugh Ennor, and included the well-regarded Secretary of the Department of Education, Mr Ken Jones, and some named but lesser fry. Yet the very possibility of some private shenanigans continued to worry some participants. The influential Academy of Science is understood to have initially refused to make a submission over the Academy's name.

As far as the astronomical politicians were concerned, an IDC was quite clearly a most inappropriate vehicle for assessing their aspirations. For the government, however, it has kept the lid on some vocal people for a while. Yet the government has let itself in for at least one more round by announcing that the IDC's report (intended for the Minister for Science) would be referred to ASTEC "for comment". And ASTEC reports to the Prime Minister, who is charged in most cases, and certainly including this one, with releasing their reports.

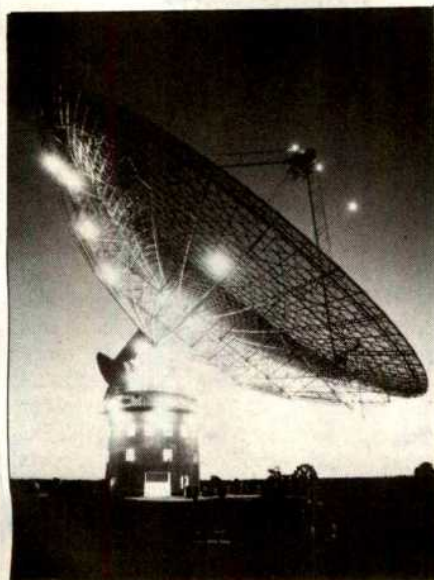
Peter Pockley

The federal Inquiry into Education and Training, which is trying to relate post-secondary education and the job market, will also impinge on science, though not directly. Professor Bruce Williams, an economist who is Vice-Chancellor of the University of Sydney, is chairing the Inquiry which is being taken very seriously indeed by the three tiers of universities, colleges of advanced education, and technical and further education.

If the current political cry for 'relevance in education' (that is less unemployment among secondary and tertiary education leavers) has any pull left by the time the Inquiry reports next year, then a reduction in the research and scholarship activity at universities relative to vocationally oriented training is possible. However, the appointment of a resilient university man as chairman is reasonable protection for the university position.



Siding Spring Observatory (see opposite)



Parkes radiotelescope (see opposite)

(continued from page 476)

ASTEC about its continuation*. Mr Fraser's statement is worth quoting at some length:

The functions of ASTEC are to advise the Government on science and technology, including

- the advancement of scientific knowledge and the development and application of science and technology in relation to the national well being;
- the adequacy, effectiveness and overall balance in the national effort in science and technology in Government, industry, education and other sectors of the community;
- the assessment of gaps and overlaps in science and technology in Australia;
- the identification and support of new ideas of science and technology likely to be of national importance;
- the practical development and application of research discoveries and the fostering of technological innovation in industry; and
- the means of improving efficiency in the use of resources related to science and technology.

The Council will have a strategic role in assisting the Government to encourage Australian science and technology to meet the nation's needs and objectives. It will have no executive responsibilities but will be able to advise on operational arrangements.

ASTEC's knowledge and analysis of science and technology will be valuable to many arms of Government. And the Government expects the Council to inform itself and be informed of relevant Government policies and to take into consideration economic and budgetary implications in discharging its functions.

ASTEC will draw on existing Departments and Agencies for the expertise, knowledge and assistance necessary to enable these functions to be discharged effectively. But this will in no way compromise the independence of ASTEC.

ASTEC has been charged first with preparing a long-overdue report on the present state of science and technology in Australia. Information about Australia's effort has been so fragmented, particularly in the government arena which dominates national R&D expenditure, that the very thought of deriving coherent policies from such information boggles the mind. This task may well be measured in years and is almost certain to out-run the life of the present Parliament, which has only 18 more months at the most.

ASTEC is thus unlikely to influence the science funding sections of the August 1977 budget, framing for which is already well advanced (to cries of "horror budget cuts"), and may hardly have sufficient time to influence the

1978 budget and the present government's final burst of legislation before facing the people again.

As anticipated in earlier reports, the permanent ASTEC reports to the Prime Minister. Its small secretariat is established within the Prime Minister's own Department. That these moves will be popular among scientists and technologists is quite certain. An analysis of the views expressed in submissions to the Interim ASTEC showed that 46 of them favoured ASTEC reporting to the Prime Minister, 6 to a Ministerial Committee, 3 to Cabinet as a whole, 4 'others', and a bare 2 to the Minister for Science. Hardly a resounding vote of confidence in the political clout of the scientists' 'own' Ministry.

The government intends that ASTEC's reports will be made public "unless there are overwhelming reasons in the national interest for not doing so" (in other words, when the security boys cry stop). Meanwhile the Australian Academy of Science, whose influence over government went into a small, temporary decline under Labor, has emerged triumphant with the appointment of its current president, Professor Geoff Badger, as part-time Chairman of ASTEC, and its immediate past president, Professor Sir Rutherford Robertson, as Deputy Chairman. Professor Badger, an organic chemist, is now a research professor at the University of Adelaide, having recently completed a ten-year stint as its Vice-Chancellor. He is firm, persuasive and skilled at the kind of politicking ASTEC needs for success.

While it was officially said that members of ASTEC are not selected as representatives of particular interest groups, it is not hard to see the influence on the present government of the major areas of Australian industry. The rural, steel, mining, chemical, electronics and paper industries all have senior executives on ASTEC, including three managing directors. Just how much time such busy men (there are no women among ASTEC's fifteen members) can devote to the detailed collection and assessment of information across the whole spectrum of science remains to be seen.

In theory, there appear to be few restrictions on ASTEC's coverage of the science and technology scene. The Interim Council's report set itself against covering the social and behavioural sciences, but argued for the inclusion of both the defence and health sciences. In Labor's terms of reference for an earlier Interim ASTEC, social science was in, but defence and health were out. Whether these items will be specified in the legislation is not known as yet. □

*Future Arrangements for an Australian Science and Technology Council, Report to the Prime Minister by the Interim ASTEC, November 1976 (Australian Government Publishing Service; 1977).

correspondence

Scientists think too

SIR,—Thank you for referring to my recent Presidential Address to the American Physical Society (28 April, page 759). However the irony implicit in your references to my address does not escape me. I disagree with you especially when you closely couple some of my words to "the recent frenzy to project science as still valuable".

Come off it. On rereading my address and listening to a recording I find nothing frenetic about it. In fact I delivered the address in an old sweater. I figured that a President of the American Physical Society, nicknamed Willy, was not to be outdone by a President of the United States, named Jimmy. The tape tells me I got some laughs but mainly I was in dead earnest. Sure, scientists are thinkers but our public image is just that—and long hair too! If society is to reach a solution of its present horrendous problems it must permit the scientist to be more than thinker, consultant and adviser. It must let him be a doer in the sense of executive, manager, legislator all the way to trouble-shooter and fix-it man. Society seems to have forgotten what happened in the winning of World War II and is now wasting a potent resource. You delude the public and discourage students by harping on the image of the scientist as the thinker. It obscures the role the scientist can perform as man of action.

WILLIAM A. FOWLER

Past President,
American Physical Society,
Caltech, California

SIR,—While agreeing with the sentiment of your advocacy for the role that could be played by scientists in the affairs of the nation (28 April, page 759), your suggestion that the mass media should take it upon itself to act as a public relations vehicle for science is both a serious indictment of scientists, and a misconception of the role of the media.

Science is undoubtedly the most highly funded cultural activity of today, yet precious little if any of this funding is devoted to developing the communication talents of its practitioners. British scientists, in my experience as a programme producer, are no more nor less talented as broadcasters than any other group of pro-

fessional people. They are certainly no more nor less perceptive about the affairs of the nation than say lawyers, historians, philosophers. Your proposal that the media create an image for scientists seems to be asking that the media take part in a cover-up campaign. You're asking us to hide a failure in the training of scientists.

You write that "very few scientists are allowed to express their own views in any extended way". I could quibble with your words "very few", but I would like to draw your attention to some science broadcasts you appear not to hear. For example, the fortnightly *Scientifically Speaking* on Radio 3 is the kind of programme you say is missing. In a year's broadcasts about 30 notable scientists (not to my mind "very few") take part usually in a 45 minute conversation. Through the questions of the interviewer, even the totally uninitiated yet intelligent listener is taken to the epistemological frontiers of science. Sydney Brenner, Leon van Hove, Nick Humphrey, D. T. White-side and B. F. Skinner—all participants in recent editions of *Scientifically Speaking*—falsify your general proposition that radio does badly in portraying the scientist as thinker. In broadcasting, what achieves impact is high programme standards, not saturation of the wavebands with merely adequate broadcasts mounted to present an image.

There also exists on Radios 3 and 4 another admirable vehicle for scientists as homo 'sapiens'—the scripted talk. I am not inundated with unsolicited ideas. You might well argue that we are too restrictive in allowing speakers the freedom to choose their topics. I would counter by drawing your attention to the long running Radio 3 series *Personal View*, transmitted on alternate Saturday evenings. In the course of four, 20-minute *Personal View* talks, the speaker is encouraged to reflect on topics of his own choice. Over the past three years, the three scientists who have given *Personal View* series have set a high standard for the others to come. Dauntingly, it seems, because many of those approached are put off by a brief that asks them to argue on topics that are in the public eye.

Surely scientists do have views, but why are they so often inhibited when offered a platform to expose their thoughts on issues outside their field of

professional enquiry? Let's not have image-building. Broadcasters are not public relations men; let's have the real article.

DAVID PATERSON

BBC, London, UK

T. H. Huxley, a self portrait

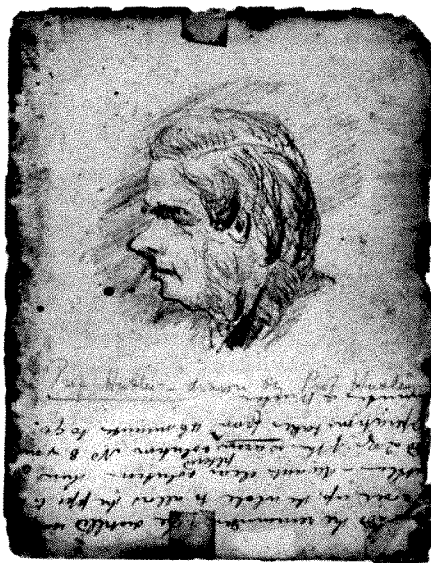
SIR,—I found this self-portrait of T. H. Huxley among the effects of Mr W. D. Bradfield, assistant to Sir William de W. Abney, a former physics professor at Imperial College, best known for his researches in colour vision.

In his autobiography T. H. Huxley states that he inherited his faculty for drawing from his father but never received any formal training. Sir Julian Huxley considered his grandfather's sketches almost as finely executed as those of Leonardo da Vinci.

Bradfield photographed the drawing and the back of the photograph reads "photograph of pencil drawing of Professor Huxley. Drawn by Professor Huxley in Captain Abney's memo book". Abney joined the Science and Art Department, South Kensington in 1877 and Huxley resigned his appointment at the Royal School of Mines in 1885. The sketch was therefore probably drawn sometime during this period when Huxley was in his mid-fifties.

M. DAVIES

University of Surrey,
Guildford, UK



news and views

The protein explosion

from Denis Bray

THOSE cell biologists who feel that the flux of information in their subject is already too high will view the latest development in gel technology with mixed feelings. For where the typical SDS gel of two years ago had some 20 well resolved bands, the two-dimensional gels that are presently *de rigueur* for the analysis of protein mixtures have several hundred. The amount of information obtained from one sample is thus appropriately, squared. The new method, devised by O'Farrell (*J. biol. Chem.* **250**, 4007; 1975), consists in electrophoresis in the presence of urea and ampholines in one dimension, and in SDS in the second. The proteins move into positions in a two-dimensional array determined by the independent parameters of isoelectric point and polypeptide size.

The system is potentially able to resolve 7,000 proteins, and although in his paper O'Farrell modestly admitted to having achieved only 1,100 from a bacterial lysate, the limitation is more likely to be in the *E. coli* genome than in his technique. From the rapidity with which it has been exploited the method obviously satisfies a long-felt need, and for many purposes it replaces a number of more complicated methods in which protein bands from gels are identified by proteolytic or chemical cleavage. It has already been widely applied and has shown even familiar biological materials to be more complicated than anyone had imagined—the flagellar axoneme for example, has over 100 components (Piperno *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 1600; 1977). The worry that the electrofocusing step might generate artefactual spots may have existed at the early stages but has gradually disappeared with use. It is explicitly disproved by such striking demonstrations as that of Milman *et al. (Proc. natn. Acad. Sci. U.S.A.* **73**, 4589; 1976), that a single genetic defect in HeLa cells affects only one spot on a 2-D gel. One is forced to conclude that each spot corresponds to one protein, and in consequence that

there are a very large number of proteins about which we know nothing at all.

Another, ostensibly unrelated, way to examine proteins is with fluorescent antibodies. This established procedure, developed by Coons more than 35 years ago, received its cell-motility debut quite recently with the isolation of anti-actin and its use to show microfilament bundles within fibroblasts (Lazarides & Weber *Proc. natn. Acad. Sci. U.S.A.* **71**, 2268; 1974). Since then, chorus-lines of glittering actin, myosin, filament protein, or LETS protein—to name a few—have brightened the biological journals in such routines as mitosis, transformation or colchicine treatment. Each pattern is characteristic of the protein and situation, even—according to the provocative suggestion of Albrecht-Bühler (*J. Cell Biol.* **72**, 595; 1977)—to the point of showing familial resemblances between daughter fibroblasts. The technique is not restricted to major protein components; indeed, because its sensitivity is uncontrollable even trace contaminants can give a sizeable response. Just how sensitive the method is will depend on the precise conditions, but some idea may be gained from the fact that a few hundred thousand antigen molecules on a lymphocyte surface give an adequate response. From this it should be possible to see 1,000 molecules of an antigen on a small area.

The two approaches of gel electrophoresis and immunological localisation have often been combined, in two recent reports of elegant work on muscle for example. Antibodies to C-protein (named for its position on an SDS gel) were prepared by Craig and Offer (*Proc. R. Soc. B* **192**, 439; 1976), and found to bind nine transverse stripes on either side of the A-band of striated myofibrils. That is, nine stripes were labelled in fibrils close to the edge of the muscle fibre, but only seven or eight in fibrils closer to the centre. The difference was revealed as being due to two other antigens in the original C-protein mixture, and by absorption, one or two stripes of the original nine could be stained. An even closer relationship between the techniques exists in recent

work on the 100 Å filaments of smooth muscle. These were recently prepared in good yield by Cooke (*J. Cell Biol.* **68**, 539; 1976), and by Small and Sobieszek (*J. Cell Sci.* **23**, 243; 1977), and are made of a protein with an apparent molecular weight on SDS gels of about 50,000. If this band is excised and the protein it contains injected into a rabbit, antibodies are obtained that stain not only smooth muscle, but the Z-disc and inter-fibrillar regions of striated muscle, and the areas of cell-to-cell contact in heart (Lazarides & Hubbard *Proc. natn. Acad. Sci. U.S.A.* **73**, 4344; 1976). When it is examined on 2-D gels this protein reveals two components which seem to be the same regardless of the muscle type. (Izant & Lazarides *Proc. natn. Acad. Sci. U.S.A.* **74**, 1450; 1977).

The synergism between gels and antibodies illustrated by such work is impressive but short of the ultimate. For surely it is entirely feasible now to extract proteins from large 2-D gels, raise antibodies to them, and use these to locate and purify the native protein? The possibilities then become awesome. If a technique in which a thousand proteins can be resolved is combined with one that can detect a thousand molecules, it could be like bringing two sub-critical masses of fissionable material together. What then would limit the isolation of new proteins, and how many biochemists would have to be applied, and for how many years, before the end was reached? Perhaps it will depend, ultimately, on how many proteins there are in all. The thousand spots seen by O'Farrell in extracts of *E. coli* must have represented the lion's share of that organism's proteins and if mammalian cells have of the order of 10,000 proteins—in keeping with the genetical estimates of 5,000 for *Drosophila* and 2,000 for *Caenorhabditis*—then there will be some hope of a complete account. But what if the geneticists are wrong and most of the potential for 100,000 proteins in the non-repetitious DNA is expressed? And what if someone invents a 3-D gel? Then, surely, it will be time to call a halt and to show the responsible attitude recently developed towards plas-

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mid research. Perhaps the discovery of new proteins could be confined to rigorously controlled (P4) conditions, or a two-year moratorium on gels could be held. At least that would let us catch up with the literature. □

Metabolic studies using ^{31}P NMR

from G. E. Chapman

THE state of proton NMR spectroscopy of biological macromolecules is somewhat akin to that of X-ray crystallography before methods of solving the phase problem had been devised. There is an embarrassment of detail, with as yet no reliable methods of interpreting it in terms of molecular conformation, though it is relatively simple to work backwards from a known structure to the observed spectrum. It is not surprising therefore that much effort is now being directed towards the NMR study of those heavier nuclei where there is less detail in the spectra, and interpretation is easier.

The increased use of ^{31}P NMR in biological research in the past year or two owes much to magnet development. In the continuing search for greater sensitivity, the NMR spectrometer designer has usually opted for higher magnetic field strengths, stretching superconducting magnet technology to the limit. However, there are indications that increasing magnetic field strengths are not achieving increases in sensitivity for the heavier nuclei when they are incorporated in macromolecules. Hence one alternative development has been that of magnets capable of maintaining very homogeneous fields over large sample volumes. This is particularly advantageous where the sensitivity is limited by the concentration of the sample rather than by the amount of material available. The following recent work has been made possible by these developments.

The monitoring of phosphate metabolite levels in living cells and tissues by NMR, being non-destructive, is aesthetically more pleasing than the conventional freeze-clamp techniques that have been used in the past by biochemists. However, it could be argued that both methods give the same results, and that a great many freeze-clamp experiments can be done for the price of buying and maintaining a modern advanced NMR spectrometer.

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This question probably cannot be resolved at present. A hybrid of the two methods has recently been illustrated, where the phosphate metabolites from the extract of a quick-frozen heart were analysed by ^{31}P NMR (Garlick *et al.* *Biochem. biophys. Res. Commun.* **74**, 1256; 1977).

One type of measurement by ^{31}P NMR in living cells however is giving results which have not been obtained reliably and accurately by other methods. This is the measurement of intracellular pH. It is achieved by observation of the inorganic phosphate chemical shift, which changes with pH in the region of neutrality due to its second acidic dissociation, and is capable of considerable accuracy (better than ± 0.02 pH units near pH 7). The intracellular pH of living *Escherichia coli* suspensions has been measured in a variety of metabolic conditions (Navon *et al.* *Proc. natn. Acad. Sci. U.S.A.* **74**, 888; 1977). It was shown that the intracellular pH was consistently higher than that of the external medium during respiration, but if respiration ceased, then the pH values rapidly equalised. This supports the hypothesis that an outward directed proton pump mechanism operates during respiration. Experiments on chromaffin granules (the catecholamine-storing vesicles of the adrenal medulla) by ^{31}P NMR also used chemical shifts to detect pH changes within the vesicles (Casey *et al.* *Biochemistry* **16**, 972; 1977) though in this case the resonance monitored was the γ phosphate of ATP rather than inorganic phosphate.

Although *in vivo* experiments are inherently more spectacular than *in vitro* ones, from the view point of the biologist, the latter type of study can be better controlled. Investigations of the important metabolic regulatory enzyme system glycogen phosphorylase, is a classic example of the importance of *in vitro* studies of metabolic processes. There are at least five phosphate compounds associated with the enzyme as substrate, cofactors and allosteric effectors. It has been investigated by ^{31}P NMR in the past, but the high molecular weight has meant the use of very high protein concentrations to achieve adequate molar concentrations of the bound phosphates, which in turn has given rise to considerable viscous broadening of the resonances. This has recently been overcome by the use of large sample volumes, and in the latest paper (Feldmann & Hull *Proc. natn. Acad. Sci. U.S.A.* **74**, 856; 1977) the role of pyridoxal phosphate in the enzyme complex has been investigated. In such an NMR experiment, one would expect some of the resonances to overlap, and the authors overcome

this by the ingenious device of using thiophosphate analogues, where these were known to be active in the enzyme system. Thiophosphates have chemical shifts about 40 p.p.m. downfield from those of the corresponding phosphate compounds. Hence the resonances of all the phosphates could be resolved. In this way, much was learnt about the interaction of the molecular species involved.

The above exemplifies, I believe, how good communication and teamwork between the biochemists and physical technique specialists can advance the application of a technique to biological problems. Here the interdisciplinary approach is paying handsome dividends. □

The nucleotide-binding fold revisited

from C. C. F. Blake

SOME time ago in these columns (*Nature* **250**, 284; 1974) I discussed the significance of a particular protein fold that had been noticed by Rossmann to constitute part or all of the structures of four NAD-dependent dehydrogenases and a number of other enzymes, some but not all of which also bind nucleotides. In lactate-, alcohol-, and glyceraldehyde-3-phosphate dehydrogenase, the fold corresponds to a separately organised NAD-binding domain, that in each molecule is linked to a second domain whose tertiary structure is specific to the particular enzyme. On the basis of this structural pattern Rossmann and his colleagues (*Nature* **250**, 194; 1974) proposed that the NAD-binding domains had evolved by divergence from a common ancestor, and had been incorporated into the dehydrogenases by gene fusion with another protein fragment, different in each enzyme, that became responsible for that enzyme's particular catalytic and substrate binding properties. Potentially this proposal provides a profound insight into the evolution of those groups of enzymes that catalyse the same type of reaction, often using common cofactors, but working on quite different substrates. The key to the proposal is the assumption that an extensive structural similarity, unsupported by sequence homology, represents the relic of a very distant gene duplication. However, so little is known about the factors controlling protein

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structures that it is very difficult to draw the essential distinction between homology and analogy. Since in the past few months three papers have appeared that in various ways bear on the problem of the significance of the nucleotide-binding fold, it seems worthwhile to return to the topic.

Describing the tertiary structure of phosphorylase α , Fletterick *et al.* (*J. biol. Chem.* **251**, 6142; 1976) note that a part of its 800-residue polypeptide chain is folded into the nucleotide-binding pattern, and demonstrate its close similarity to the NAD-binding domain of lactate dehydrogenase. In agreement with the prediction of Rossmann's proposal, Madsen and his colleagues have observed that the fold is the location of a binding site for adenine or adenosine that is close to the site for the adenine ring of NAD in the equivalent region of lactate dehydrogenase. Unfortunately the significance of this binding site is not known, and the site for the known allosteric effector AMP, is elsewhere in a region whose structure is quite different from the nucleotide fold. Nevertheless, the discovery of the fold in yet another enzyme, associated with nucleotide binding, must strengthen the hypothesis considerably.

One of the features of the fold that was instrumental in its discovery was that the usually helical loops that sequentially link together the six-stranded parallel β sheet, were all arranged in a right-handed sense, thus giving rise to a characteristic distribution of connections above and below the sheet. At the time both right- and left-handed turns were thought to be allowed, suggesting that the repeated occurrence of a particular pattern in the disposition of the connections was highly significant. Sternberg and Thornton (*J. molec. Biol.* **105**, 367; 1976) have now shown that this view is incorrect. By analysing all known proteins in terms of the disposition of the loops linking adjacent β strands, they have found that in 57 out of 58 cases the connection has a right-handed sense. Furthermore they have produced a soundly based argument that the right-handed connection is a natural consequence of the steric factors imposed by the twist of the β sheet, which Chothia (*J. molec. Biol.* **75**, 1295; 1973) has shown, can itself be understood in terms of the interactions within the individual strands of the sheet. This means that the five right-handed connections in the nucleotide-binding fold are not independent parameters, with which the fold can be defined, but are the consequence of good molecular packing. The removal of these parameters has dramatically reduced the figure of topological relatedness calculated by Schulz and

Schirmer (*Nature* **250**, 142; 1974) for a number of proteins in which the fold seemed to occur, to leave only the topologies of the cofactor-binding units of the dehydrogenases with a sufficient degree of relatedness to need a special explanation. Rossmann's hypothesis still stands then, stronger I suspect for having a number of dubiously-related enzymes stripped away from it by Sternberg and Thornton, leaving a solid core of structure whose relations have a firm functional foundation.

The most striking new addition to the catalogue of enzymes showing tertiary structural homology is pyruvate kinase, whose structure has been recently reported by Stammers and Muirhead (*J. molec. Biol.* **112**, 309; 1977). The structure of the 60,000 molecular weight subunit is divided into three domains, the largest of which is closely similar to the complete triose phosphate isomerase (TIM) subunit. As described by Banner *et al.* (*Nature* **255**, 609; 1975) the TIM subunit has a hitherto unique structure composed of eight parallel β strands forming a closed barrel in the core of the molecule, surrounded by helical connections, giving it an unusually simple, symmetrical organisation. This structure is sufficiently characteristic for there to be little doubt that it constitutes the central domain of pyruvate kinase. The active site of pyruvate kinase is located in an equivalent position to the active site of TIM, in relation to the common structure. These structural and functional similarities between pyruvate kinase and TIM are of the same order as those in the various versions of the nucleotide-binding fold, and should therefore in principle be treated in the same way. Is it then plausible that pyruvate kinase and TIM should have a common ancestor? The only thing that one can say is that despite the quite different kind of reaction catalysed by the two enzymes, their substrates are both 2-keto-trioses. Whatever arguments are applied to the significance of the nucleotide-binding fold; it seems that they must now also bear on the 'keto-triose fold' and *vice versa*; they stand, or fall, together.

Finally the structure of pyruvate kinase focuses attention on the very curious fact that while the four dehydrogenases whose tertiary structures are known show a significant degree of similarity to one another, the four kinases do not. Adenylate kinase is somewhat similar to a part of phosphoglycerate kinase (PGK), but does not seem to be significantly related; PGK itself almost certainly contains at least one, if not two, copies of the nucleotide-binding fold; pyruvate kinase is structurally related to TIM as we have seen; and hexokinase seems

to have a unique structure. The search for indications of enzyme families through structural similarities will continue, but it seems that any pattern that emerges is unlikely to be simple and clearcut. $\square$

Systematic behaviour of E1 decays in sodium

from P. E. Hodgson

THE electric dipole (E1) transitions in light nuclei have so far received little theoretical attention because selection rules often forbid transitions between the major components of the wavefunctions of the initial and final states, so that they depend on small components of the wavefunctions which are difficult to calculate. In spite of this, the experimental E1 transitions in ^{23}Na show a remarkably simple pattern that is quite different from that given by the simple rotational model. Recently Pilt has developed an alternative model that agrees very well with the experimental data for ^{23}Na and also for ^{21}Na . (*Z. Naturforsch* **32a**, 73; 1977).

The E1 decays in ^{23}Na take place between the lowest $1/2^-$ band and the ground state $3/2^+$ band. It is found that when the overall E^3 , dependence of the transition rates is removed a spin J state of the $1/2^-$ band decays preferentially to a spin $J+1$ state of the $3/2^+$ band. A completely different behaviour is predicted by the simple rotational model, which gives reduced transition strengths proportional to the squares of the appropriate Clebsch-Gordan coefficients.

It is most likely that such regular deviations from the simple rotational model are due to an interference effect, and Pilt suggests that this can be provided by mixing higher Nilsson orbitals into the ground state band. The most likely orbitals are those with spin and parity $1/2^+$ and those with the configurations [220] and [211] are the most probable. The E1 transition rate is then given by the absolute modulus squared of the sum of the appropriate Clebsch-Gordan coefficients, multiplied by weight factors, that is,

$$B(E1) = |(J/2 \ 11/J'3/2) + m_1(J/2 \ 10/J'1/2) + (-)^{J'-1} m_2(J/2 \ 1-1/J'-1/2)|^2,$$

where J and J' are the spins of the initial and final states, and m_1 and m_2 are the weight parameters which are related to

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the degree of mixing between the $1/2^+$ and $3/2^+$ bands.

So far it has not been possible to calculate the mixing parameters m_1 and m_2 , but the model may be tested by using the experimental values of $B(E1)$ for two transitions to determine m_1 and m_2 , and then to use these values to calculate the $B(E1)$ values for the other transitions. Pilt did this for the transitions in ^{23}Na , and found that the reduced transition rates for the whole band are very well given by this model. The same analysis was applied to the transitions in ^{21}Na , and this also gave good overall agreement with the data, although it did not have such a simple pattern as that for ^{23}Na .

An attempt to calculate the mixing parameters from the Nilsson model was not successful; it was found that there is no value of the nuclear deformation that gives the observed distribution of transition strengths. It thus seems that a more detailed microscopic theory is needed to obtain a proper understanding of these quantities.

Even in its present form, with the parameters treated as adjustable and fitted to the data for each nucleus, the model can be very useful in correlating experimental data, and it could well be a useful method of determining the spins of high-spin states. It would clearly be useful to test its applicability to other nuclei, and this might show some systematic behaviour of the mixing parameters, which in turn might provide a clue to their microscopic interpretation. □

A tribe of mimics

From a Correspondent

BLENNIES are generally known as small, rather retiring fishes living in shallow water close to rocks or coral. Very many species are known in temperate and tropical seas but the latter contains the greatest number of species. A few are found in freshwater. Partly because there are so many nominal species, ichthyologists were exercised in attempting to assess their phylogeny, and for long the standard work on the group as a whole was J. R. Norman's provisional synopsis (*Ann. Mag. nat. Hist.*, ser. 11, 10, 793–812; 1943). More recently, V. G. Springer's study of the osteology and classification of the family (*Bull. U.S. natn. Mus.* 284, 1–85; 1968) has provided a major clarification and a useful overall classification within which Springer, and others, can tackle the smaller groups in the family.

The most recent is a revision of the sabre-toothed blennies of the tribe Nemophini by W. F. Smith-Vaniz (*Acad. natn. Sci. Philadelphia, Monogr.*

Origin of North China loess

from I. J. Smalley

CHINESE civilisation developed on the great North China loess deposits. Ho (*Cradle of the East*, Chicago University Press, 1975) has argued convincingly that this development took place in isolation, uninfluenced by the parallel progress in the Middle East. A major factor which did influence the growth of the early Chinese civilisation was the presence of the loess which was an ideal soil for the early farmers. The origin of this North China loess is still in dispute. Most of the other loess deposits in the world have glacial connections and their formation can be seen as part of a sequence of Pleistocene events, but the Chinese loess does not have an obvious glacial link.

The results of the first electron microscope observations of Chinese loess have now been published (Lu *et al. Geochimica* 47, 1976). These allow the Chinese workers to reject totally the Berg theory of *in situ* formation, but they still find themselves unable to accept totally a direct glacial connection. The grains observed are broken and have sharp edges, and silica precipitation and chemical etching are commonly observed on the quartz silt particles, which might have been subjected to relatively long weathering processes

before being transported to the loess accumulation areas. In the relevant areas the authors suggest that glaciation only occurred on mountain tops, and that this may have produced some loess, but they favour a distant western source for the majority of the material. No specific particle-producing mechanism is described.

It may be that in this case a longer than usual transportation stage was involved and that material was formed in distant glaciated regions and that the continuing aridity and the presence of mixed particle deposits in the post-Pleistocene times prevented the formation of stable loess deposits. In Europe and North America loess deposits form very close to the ice limits. The Lu *et al.* paper is an encouraging sign of the renewal of interest in loess sedimentology in China. It is a pity that those otherwise admirable data handlers, *Science Citation Index* and *Mineralogical Abstracts*, have not realised that in Chinese the family name (as used in references) comes first; so this important paper has been listed under Yanchou *et al.* which could cause some confusion.

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19, 1–196; 1976); an excellent contribution to the systematics of these Indo-Pacific blennies. As a taxonomic work it is of fundamental importance for not only are 41 species in 5 genera fully described and figured, but 13 of these species are new taxa, recognised here for the first time. However, this paper is important not just because it is a highly competent revision, but because of the information about the life styles and behavioural relationships of this fascinating group of blennies.

The sabre-toothed blennies are so called because they all have a massive recurved canine tooth posteriorly in the lower jaw. Most species also have smaller caniniform teeth in the upper jaw. In the front of both jaws the teeth are small, closely-packed and incisiform. Such dentition suggests that their feeding behaviour might be specialised, an expectation which is fully realised.

Members of the genus *Meiacanthus* are unique among fishes in having well-developed toxic buccal glands. Their sabre teeth are grooved, the groove leading to the gland in the dentary bone and acting as a channel for the pale, toxic secretion. All the species of *Meiacanthus* bite when attacked, and

experiments have shown that they are generally unacceptable as prey although naive fish predators may take them into their mouth, only to spit them out quickly. In ecology and behaviour they differ strikingly from most blennies. They inhabit open water and in daytime forage over a wide area in search of small crustaceans in the water column, and tend to hover in one spot then jerk away, to hover again, feeding as they pause.

Their virtual immunity from predators no doubt has a bearing on their behaviour and it is not surprising that several species are brilliantly coloured in life. Various *Meiacanthus* species have close mimics, including unrelated fishes, as well as other sabre-toothed blennies.

The latter include several species of *Plagiotremus* which hover and jump in much the same way as the *Meiacanthus* model. The former feed on larger fishes, especially non-predatory ones, hovering nearby and then launching a lightning attack on the passing fish, usually from the rear. If the prey turns head-on suddenly the attack will be aborted. Their incisor teeth are curved, sharp in the horizontal plane, but flattened in the lateral, are well adapted

to slice skin and scales out of their prey, while the massive fangs provide a purchase as well as breaking the surface tissues. In all the specimens which contained food, Smith-Vaniz found mucus and dermal tissue.

A member of a third genus of sabretoothed blenny, *Aspidontus*, is also an accomplished mimic, but this time of the cleaner wrasse, *Labroides dimidiatus*. *Labroides* is cryptically coloured and tends to hide when approached. *Aspidontus taeniatus* employs its resemblance to the generally unmolested cleaner wrasse to approach naïve prey and attack them, biting off fin rays and associated membranes.

Petrosciurus, the fourth genus of sabretoothed blenny also includes at least two mimics of the venom-fanged *Meiacanthus* species, but most of the 10 species recognised by Smith-Vaniz are retiring and cryptically coloured. They feed on copepods and diatoms and are thus rather unremarkable as

these blennies go. However, for small fish (they rarely grow longer than 60 mm in body length) they are well able to defend themselves, even by snapping at probing fingers.

To complete his account of the nemophine blennies, Smith-Vaniz describes the two species of *Xiphiasia*. True to the nonconformist nature of the tribe, they differ from most blennies. They may attain a length greater than 500 mm, so they are exceptionally large members of the family. They are also semi-pelagic and probably nocturnal, and it seems that they burrow tail first into sand or mud, but their biology is not yet fully studied.

Smith-Vaniz's revision of this tribe of blennies, although primarily a taxonomic treatment, shows them to be animals of striking behavioural and ecological divergence. This group, at least, are far from the dull-coloured, retiring, and modest animals that one generally thinks of as blennies. □

Washington, 1975)). At this stage active transport into the spore compartment can no longer take place so that perhaps exogenous gramicidin is unable to exert its effect.

The requirement for gramicidin at a specific time well before the acquisition of heat resistance suggests that the antibiotic has an indirect effect. More recent work from the same laboratory (Sarkhar, Langley & Paulus *Proc. natn. Acad. Sci. U.S.A.* **74**, 1478; 1977) indicates that gramicidin is a potent and specific inhibitor of transcription *in vitro*. This contrasts with the assumption made hitherto that the antibiotic increases the permeability of cell membranes (Sadoff *Spores* **V**, 157 (American Society for Microbiology, Washington, 1972)). This new work, which made use of a cell-free transcription system devoid of membrane, shows that gramicidin inhibits binding of RNA polymerase to DNA. The inhibition seems quite specific for certain regions of the DNA and this led the authors to suggest that the antibiotic specifically inhibits transcription of particular genes; perhaps those concerned with vegetative functions but not with sporulation. In all work of this kind, however, it is difficult to determine *in vivo* effects of a compound since observations made *in vitro* may be inapplicable to the cells' physiological conditions.

The studies of Paulus and coworkers show that gramicidin is unnecessary for the formation of functional spores though in its absence the spores have some altered properties. This is consistent with the idea that gramicidin acts at a time when it is possibly being produced for a quite different reason—as an antibiotic, for example. Such an interpretation is compatible with the

Antibiotics and bacterial sporulation

from Brian N. Dancer

It has been known for many years that bacteria of the genus *Bacillus* produce antibiotic substances when the cells are beginning endospore formation at the end of growth. These compounds consist of oligopeptides often containing non-protein amino acids and they are not synthesised on ribosomes. They are a heterogeneous group of compounds some of which (such as gramicidin) are surface-active agents whereas others (bacitracin for example) inhibit cell wall synthesis. For some time there has been controversy over their function. Secretion at the end of growth has been seen as a mechanism for inhibiting competitors, and allowing greater growth by the producer organism. Alternatively, antibiotics may be secondary metabolites synthesised to remove accumulated toxic compounds. A third view is that their production at the beginning of sporulation indicates a role in the regulation of the developmental process. If the last were true one would expect that cells deficient in antibiotic synthesis would sporulate abnormally. This hypothesis has recently been tested by Mukherjee and Paulus (*Proc. natn. Acad. Sci. U.S.A.* **74**, 780; 1977) in *Bacillus brevis*, a strain which produces two classes of antibiotic, gramicidins and tyrocidines. The authors were able to select mutants using an ingenious technique developed

by Raetz (*Proc. natn. Acad. Sci. U.S.A.* **72**, 2274; 1975). These mutants are unable to make gramicidin although tyrocidine is still produced. They are able to sporulate but the spores are somewhat less heat resistant than those of the parental strain. The mutants produce reduced amounts of dipicolinic acid—a spore-specific compound.

The heat resistance of the mutant spores can be partially restored by addition of gramicidin at the end of the growth phase (when sporulation begins) but not later. This result indicates that the mutant's behaviour is caused by lack of gramicidin and not by some pleiotropic change affecting both sporulation and antibiotic synthesis. If this interpretation is correct this is the first time that a direct relationship has been clearly shown between these two processes. It is noteworthy that although gramicidin is required early in sporulation its effect is exerted on a very late event. In its absence spores are still produced (albeit slightly defective) indicating that gramicidin is not essential for a major part of the developmental process.

It is possible that the insensitivity to gramicidin after sporulation has begun is associated with an inability to take up the compound. This type of behaviour has been correlated with the closure of the double prespore membrane, resulting in two membranes of opposite orientation (Cooney, Freese & Freese, *Spores* **VI**, 187 (American Society for Microbiology,



A hundred years ago

A WONDERFUL white aquamarine has been found in Perthshire which, when cut, has produced one of the most brilliant gems ever seen. It is said by many competent judges to be equal to her Majesty's celebrated Koh-i-noor, its refraction being very great both by day and night. It is of a pure pellucid liquid white, and is known as the Scotch Koh-i-noor. Its hardness is 8.0, and specific gravity 2.76. Mr. Bryce M. Wright, F.R.G.S., is its possessor.

From *Nature* 16, 7 June, 237; 1877.

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fact that other species of *Bacillus* produce different antibiotics (unrelated chemically and in their mode of action) and yet can still sporulate normally. Although gramicidin must be considered as peculiar to *B. brevis* its properties suggest modifying functions that may apply to antibiotics in other bacteria.

This latest work from Paulus's laboratory, and in particular the technique of mutagenesis, suggests useful methods for exploring the possible function of antibiotics in other strains of *Bacillus*. It may then be possible to decide, species by species, whether these compounds are products associated with prolongation of growth or have a role in the control of bacterial morphogenesis. □

Twenty years after

from P. Pitha

Some 150 scientists attended the Fifth Aharon Katzir-Katchalsky Conference held at the Weizmann Institute of Science, Rehovot, Israel on 2-6 May, 1977. The meeting, organised by the Israel Academy of Sciences and Humanities, was opened by the President of the State of Israel, Dr H. E. Ephraim Katzir, who chaired the first session. It was a welcome, although uncommon, sight to see a head of state participating in a scientific meeting, and could probably only happen in Israel, where the president has always been a scientist.

TWENTY years after its discovery by Isaacs and Lindenmann, how far has interferon fulfilled its clinical potential? Well-controlled clinical studies of the effect of interferon on infection with herpes zoster and hepatitis virus (T. C. Merigan, Stanford University) indicate that interferon treatment started after the onset of infection shortened the time that clinical symptoms of herpes zoster infection were apparent and suppressed virus production in patients chronically infected with hepatitis virus.

In all future trials, however, the supply of interferon may be the rate-limiting step, and a number of groups are actively developing large scale production methods for human interferon. Production of interferon by genetic engineering is also an attractive proposition and several groups have started work on what is undoubtedly

going to be a long and difficult project.

Another major handicap to clinical work has been the lack of purified interferon. Now E. Knight (Du Pont, Delaware) has purified human fibroblast interferon to homogeneity, obtaining a single polypeptide of molecular weight 20,000. However, the major clinical trials have used human leukocyte interferon which is a complex mixture containing distinct components separable by charge and size (W. Stewart, New York), none of them serologically identical with fibroblast interferon. Human lymphoblastoid interferon seems to be a mixture of both leukocyte and fibroblast interferon (J. Vilcek, New York). It is important to determine whether these differences are due to different primary structures, and hence different genes, or to differences in post-translational modification.

Several lines of evidence show that interferon is newly synthesised after induction, and not formed from an inactive precursor. Genetic analysis of human-mouse hybrids continues, although it is still not completely clear whether induction of human interferon requires two chromosomes or only one. Further characterisation of virus-resistant mutant 3T6 cells suggested that constitutive interferon production is involved in this resistance (C. Colby, New York).

It has been shown that interferon production is not regulated at the level of transcription by comparison of the mRNA levels (assayed by injection into *Xenopus* oocytes) with the amounts of interferon produced in human fibroblasts induced by poly (rI)·poly (rC) in normal and superinduction conditions (in which high yields of interferon are produced) (P. Pitha, Johns Hopkins University).

P. Marcus (University of Connecticut, Storrs) reported that a VSV defective interfering particle produced a high yield of interferon at low multiplicity, possibly by formation of a double-stranded RNA within the cell (see *Nature* 266, 815; 1977). This is the best evidence so far that viruses and polynucleotides induce through a common mechanism—formation of double-stranded RNA. However, little is yet known about how the interferon gene(s) is depressed.

Interferon acts by first interacting with the cell membrane, an interaction involving both gangliosides and glycoproteins (H. Ankel, University of Wisconsin, Milwaukee; R. Friedman, National Institutes of Health, Bethesda). Sensitivity to interferon seems to be determined by the receptor located at the cell membrane, however, it is not yet clear whether the species specificity is determined at the same level (C. Chany, Paris; M. Revel, Weizmann

Institute, Rehovot). Work with cell-free systems has made it clear that interferon produces several different effects.

Thus it was shown that in cells of different species interferon treatment induces an increase in the phosphorylation of specific polypeptides. Revel found in interferon-treated cells, the increase of a specific protein kinase which inhibits translation and is cyclic AMP independent. Both the phosphorylation and the translational inhibition mediated by protein kinase are enhanced in the presence of double-stranded RNA. A heat stable, low molecular weight translational inhibitor (probably an analogue of oligo adenylic acid) was found in interferon-treated cells by I. M. Kerr (National Institute of Medical Research, London). For the synthesis of this inhibitor both double-stranded RNA and ATP are required. However, other cellular enzymes such as an endo RNase described in detail by P. Lengyel (Yale University) may be regulated by double-stranded RNA and ATP in interferon-treated cells. The latter two systems, however, were not dependent on the phosphorylation. However, there is as yet no explanation of why the translation of viral mRNA is preferentially inhibited in the intact cell.

In the past, investigation of interferon's antiviral effect has been primarily on the small lytic viruses, and W. Joklik (Duke University, North Carolina) continues his elegant studies on reovirus. However, attention has now shifted to the oncogenic viruses. E. Winocour (Rehovot) and Revel reported that when interferon is added to cells shortly after infection with SV40, it affects translation only. Three papers (Pitha; Friedman; A. Billiau, University of Louvain) on the effect of interferon on MuLV infection showed that in contrast to all other viral systems studied, a late event in virus replication was affected, probably a stage in virus maturation and assembly.

Amounts of purified human interferon similar to those required for the antiviral effect slow cell growth (Knight). Interferon also inhibits delayed type sensitivity, affecting both sensitisation and the expression of the sensitised state (E. De Maeyer, Paris). Interferon also increases the effects of lymphochoriomeningitis virus disease in the newborn mouse, although the mechanism is not known (I. Gresser, Paris). These reports all indicate that interferon may have functions other than its antiviral effect. An important distinction has emerged between type I interferon (virus induced) and type II (induced by antigens and mitogens), and E. Falcoff (Paris) reported that stimulation of B cells produces type I, and of T cells type II interferon. □

articles

Rb/Sr ages and geological setting of ancient dykes in the Sand River area, Limpopo Mobile Belt, South Africa*

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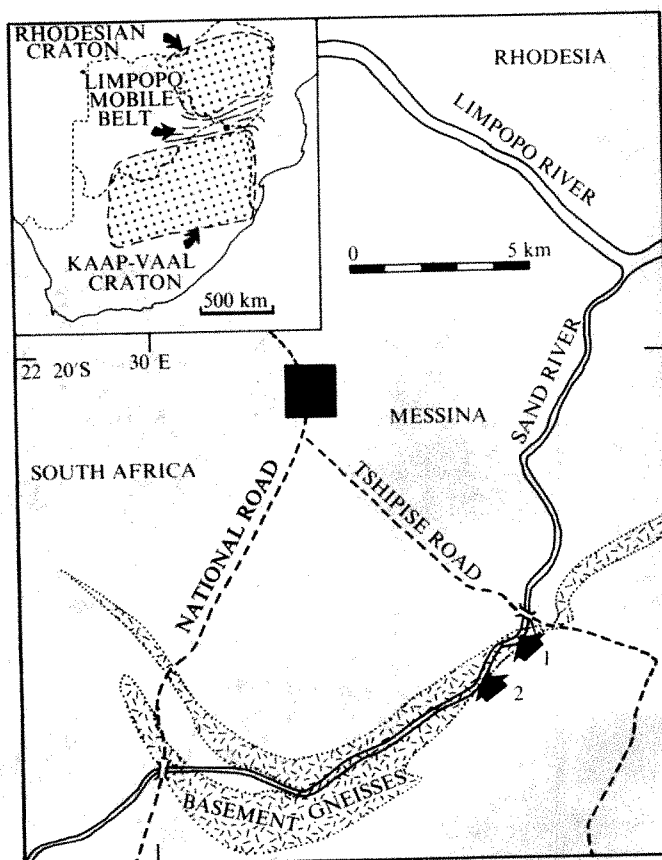
Rb/Sr whole-rock ages from two suites of tholeiitic dykes establish a pre-3,600 Myr history for some granodioritic gneisses in the Limpopo Mobile Belt and identify two previously unrecognised magmatic events in the evolution of this region. This has important implications with regard to relationships between the Belt and the adjacent Rhodesian and Kaapvaal Cratons.

WITHIN the Central Zone^{1,2} of the Limpopo Mobile Belt near Messina, South Africa, Bahnmann³ reported rocks which he considered to be basement to an overlying supracrustal sequence. A particularly spectacular exposure of both these units occurs in the valley of the Sand River (Fig. 1), where the basement rocks form a part of a structural unit, possibly a thrust and/or fold nappe, tightly infolded with the cover sequence. The presumed basement rocks, provisionally named the Sand River Gneisses, are comprised mainly of homogeneous peraluminous medium-grained quartz bearing grey gneiss and poorly banded peraluminous medium- to coarse-grained quartz bearing leucocratic gneiss. Detailed mapping (scale 1:100) at Locality 1 (Fig. 1) indicates that the basement gneisses occur as generally steep-dipping, tightly folded interlayered units showing small-scale interference patterns. In addition, both types of gneiss possess an early tectonic fabric and early quartz-feldspathic dykes and veinlets that have been folded at least twice. Rb/Sr whole-rock analyses of both of these gneisses yield an isochron of 3858 ± 116 Myr (2σ) with an initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7012 ± 0.0002 (2σ) (B.R. and J.M.B., unpublished). The rocks regarded as being supracrustal to the basement gneisses are predominantly quartzite and iron formation interlayered with garnetiferous metapelatic rocks and with two-pyroxene gneiss, presumably a metavolcanic rock. A conclusive discordant relationship between the basement rocks and the supracrustal sequence has not yet been recognised, although preliminary structural fieldwork (R.E.P.F., in progress) is consistent with such an interpretation. In particular, this work suggests that the early tectonic fabric and early quartz-feldspathic dykes found in the basement gneisses

are not present in the supracrustal rocks. No geochronological data are at present available for these supracrustal rocks.

In addition, as with similar strongly deformed and metamorphosed Archaean terrains in the Outer Hebrides⁴, West Greenland⁵ and Labrador⁶, the basement rocks contain deformed layers of amphibolite, which, in places are clearly discordant with respect to both the gneissic layering and

Fig. 1 Location map of the Sand River area showing the sampling sites. Shaded area denotes outcrops containing basement rocks.



*Contribution 9 of the South African International Geodynamics Project

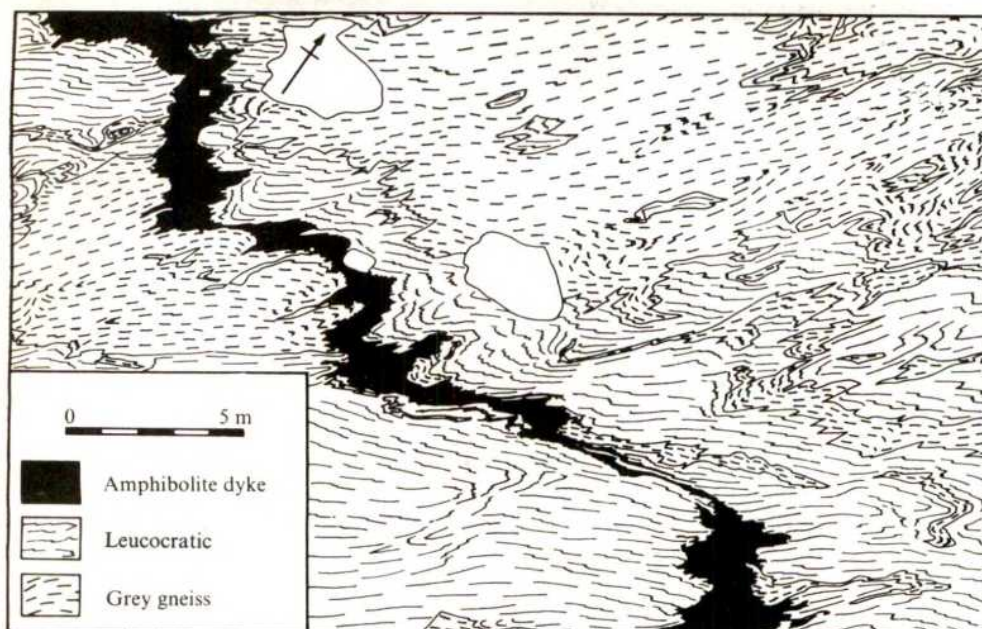
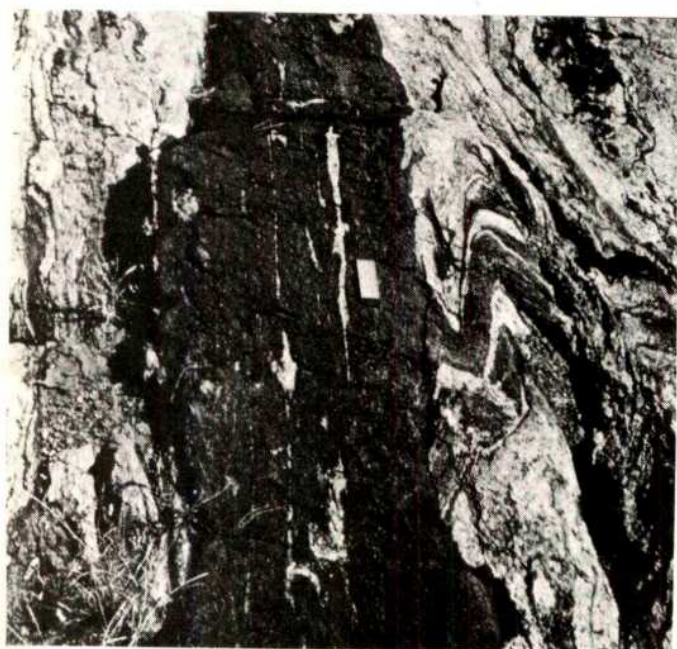


Fig. 2 Simplified geological map of a portion of the exposure of basement rocks in the Sand River area showing an older basement dyke. Blank areas are unexposed. Form lines represent pre-dyke fabric in the gneisses.

the mineral fabrics in the basement gneisses (Fig. 2) and are, therefore, interpreted as being basic dykes. These dykes post-date the early quartz-feldspathic dykes and veinlets and have been folded and metamorphosed together with the gneisses in conditions of hornblende granulite facies. They have not, as yet, been recognised within the supracrustal rocks. At Locality 2 (Fig. 1), a suite of physically similar, deformed basic dykes show discordant relations with respect to both the grey and the leucocratic gneisses and to other, evidently older, basic dykes (Fig. 3). Therefore, at least two suites of strongly deformed amphibolite dykes are recognised in the basement rocks of the Sand River area, and these are not distinguishable in the field except where juxtaposed and where original discordant relationships are preserved.

Suites of samples of amphibolite, free of younger veinlets (Fig. 3), were carefully selected for Rb/Sr whole-rock iso-

Fig. 3 Photograph of the cross-cutting relationships between the older and younger dykes in the Sand River area. The scale resting on the younger dyke is 10 cm long. Note the deformed felsic veinlets in this dyke, and the large discordant angle between the two dykes.



topic analysis (Table 1) from both the older and younger dykes. These analyses yield isochrons⁹ of 3643 ± 102 Myr (2σ) with an initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7014 ± 0.0003 (2σ) for the older suite and of 3128 ± 81 Myr (2σ) for the younger suite (Fig. 4). No clear correlation exists between the K_2O concentrations and the Rb concentrations among these samples (Table 1) and on a plot of K_2O against Rb (Fig. 5), the K_2O concentrations are largely insensitive to changes in Rb concentration for samples from each dyke suite. It is likely, therefore, that each suite underwent some post-intrusive alteration of Rb concentrations. The isochrons, consequently, must reflect the time of such alteration. In view of the older Rb/Sr whole-rock isochron of the surrounding Sand River Gneisses, and the small initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of the dyke suites themselves, these alterations were probably deuteritic in nature and involved the transfer of Rb between the wall rocks and the intruding magma. Such a process has been described by Fratta and Shaw¹⁰ and occurs before crystallisation of the dykes. The isochrons, therefore, very likely also closely approximate the time of intrusion of the dykes.

The initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of the older dykes is greater than that normally postulated for the oceanic mantle ~ 3643 Myr ago¹¹⁻¹³. On the other hand, the initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of the younger dykes corresponds closely to the mantle value for ~ 3128 Myr ago. It is possible that the higher initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of the older dykes is a consequence of crustal contamination and/or metasomatism ~ 3643 Myr ago. Such hybridisation would necessarily require either the addition of Sr with an $^{87}\text{Sr}/^{86}\text{Sr}$ ratio greater than ~ 0.7014 or the removal of Sr with an $^{87}\text{Sr}/^{86}\text{Sr}$ ratio less than ~ 0.7014 . These initial ratios, however, are also consistent with the possibility that these dykes were derived from distinct sources. If this is true, they support the suggestion by Condie¹³ that a heterogeneous mantle under continental areas formed early in Archaean times and also that a continental-type lithosphere of the type envisaged by Brooks *et al.*^{14,15} may have already been in existence in this region before ~ 3643 Myr ago.

The concept of a heterogeneous subcontinental mantle is further supported by major and trace element compositions of samples from these dykes. As far as major element chemistry is concerned, both generation of dykes are almost identical (Table 2). They have compositions of quartz-normative tholeiites showing significant iron and titanium enrichment, and they contain more iron and titanium than either the Ameralik dykes of West Greenland (13.30%

Table 1 Rb/Sr isotopic data for basic dykes

Sample	^{87}Rb (p.p.m.)	^{86}Sr (p.p.m.)	$^{87}\text{Rb}/^{86}\text{Sr}$	$^{87}\text{Sr}/^{86}\text{Sr}$	K_2O (weight %)	K/Rb
Older dykes						
B-75-57	2.64	40.8	0.0640	0.7047	0.85	742
B-76-25	1.76	11.7	0.1485	0.7090	0.93	1204
B-75-59	2.62	13.5	0.1921	0.7115	0.85	744
D2	2.97	11.0	0.2672	0.7151	0.97	770
D1	3.20	10.5	0.3013	0.7171	0.99	702
Younger dykes						
B-75-55	2.24	11.9	0.1861	0.7084	0.74	716
B-76-115	4.08	16.7	0.2416	0.7109	0.91	514
B-75-60	3.48	12.6	0.2724	0.7123	0.55	367
B-76-116	6.07	19.0	0.3150	0.7142	0.83	316
B-75-58	4.35	12.4	0.3464	0.7153	0.73	384
B-76-117	5.12	12.5	0.4058	0.7181	1.01	453
B-75-54	5.58	12.4	0.4461	0.7201	0.37	913

Twelve samples were collected for study. Of these, five samples were from demonstrably younger dykes, and four from the older dykes. The remaining three samples came from dykes which from a field standpoint, were of uncertain age. Powders of the samples were simultaneously spiked for Rb ($>99.2\%$ ^{87}Rb) and for Sr ($>99.3\%$ ^{86}Sr). ^{87}Rb and ^{86}Sr concentrations are precise to within 0.5% (1σ). The 1σ uncertainty in the $^{87}\text{Rb}/^{86}\text{Sr}$ ratio is $\pm 0.7\%$ while the 1σ uncertainty in the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio is $\pm 0.05\%$. Replicate analyses of the Eimer and Amend standard SrCO_3 yields 0.7080 ± 0.0001 (1σ). The data were tested by both the techniques of York⁷ and Brooks *et al.*⁸ to demonstrate that they scatter less than that amount predicted from experimental uncertainties alone. The ages and initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios were calculated by the technique of York⁸ using $1.39 \times 10^{-11} \text{ yr}^{-1}$ as the decay constant of ^{87}Rb . The K_2O concentrations were determined by X-ray fluorescence spectrometry and are precise to within 0.5% (1σ).

total Fe as Fe_2O_3 ; 0.86% (TiO_2)¹⁸ or the Säglekh dykes of Labrador (14.21% total Fe as Fe_2O_3 ; 1.20% TiO_2)¹⁷. Significantly, however, they have K_2O ($0.8\text{--}0.9\%$) contents greater than those characteristic of oceanic tholeiites ($<0.6\%$ K_2O)¹⁸, but consistent with the contents of K_2O in continental tholeiites (0.9% K_2O)^{19,20}. Furthermore, the older dykes have higher Sr concentrations than do the younger dykes, consistent with the possible existence of a heterogeneous source region.

The Limpopo Mobile Belt has previously been regarded as being largely of late Archaean–early Proterozoic age^{21,22}, and the emplacement of tholeiitic dykes at ~ 3643 Myr and 3128 Myr ago establishes two additional events in the crustal development of the region. Furthermore, the timing of these events supports the possibility that basement rocks to the Limpopo supracrustal rocks are exposed along the Sand River. Some of the supracrustal rocks near Messina are intruded by the Messina Layered Intrusion and are consequently believed to be older than the 3221 ± 48 Myr (2σ) Rb/Sr whole-rock isochron age of this body²³. Some of them are also presumed to be younger than the ~ 3643 Myr age reported here for the older dykes. Therefore, either the ~ 3128 -Myr old dykes should be found in the cover sequence, or the cover sequence exposed along the Sand River is younger than the cover sequence into which the Messina Layered Intrusion was emplaced. In either case, the deformation and high-grade metamorphism

which the rocks of the Limpopo Mobile Belt have undergone, must have occurred more recently than ~ 3128 Myr ago, and this deformation and metamorphism was not of sufficient intensity to obliterate the older isotopic ages.

With the exception of the rocks into which these dykes are intrusive, no other rocks have yet been reported in Southern Africa yielding Rb/Sr whole-rock ages as old as ~ 3643 Myr. Within the Kaapvaal and Rhodesian Cratons, however, granitic rocks yielding errorchrons of between ~ 3600 and ~ 3500 Myr have been reported^{24–26}, and major igneous and/or metamorphic activity occurred from ~ 3520 Myr ago²⁷ up to ~ 2700 Myr ago^{28–31}. Consequently, compared with the ages for the adjoining Rhodesian and Kaapvaal Cratons (Fig. 1), there is nothing particularly unique to the new ages recorded in the Limpopo Mobile Belt. It seems reasonable to assume, therefore, that the Limpopo Mobile Belt is floored by and otherwise contain rocks that are coeval, but not necessarily correlative with, those in the Rhodesian and/or Kaapvaal Craton.

The Lower Onverwacht komatiitic volcanic rocks in the Barberton greenstone belt were deposited sometime ~ 3500 Myr ago^{27,29,32,33} within the span of time from ~ 3643 to ~ 3221 Myr ago during which the Limpopo supracrustal

Fig. 4 Isochron plot of the Rb–Sr isotopic data from the basement dykes in the Sand River area.

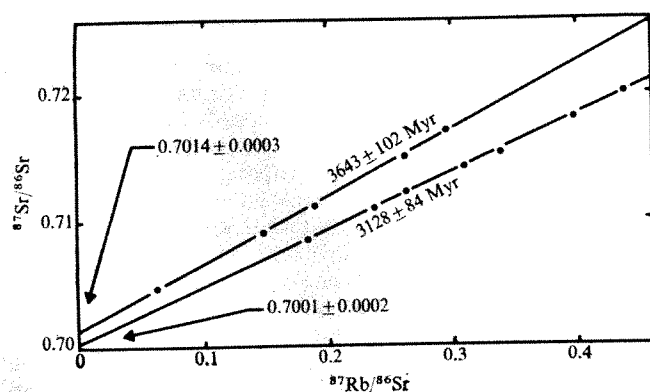
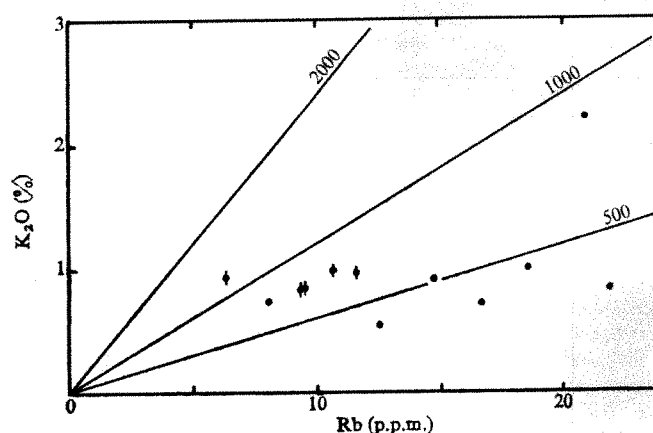
Fig. 5 Plot of K_2O (weight %) against Rb (p.p.m.) for the dyke samples analysed in this study. Lines of equal K/Rb ratios are shown for comparison. ♦, Older dyke suite; ●, younger dyke suite.

Table 2 Average chemical compositions of the basic dykes

	Older dykes*	Younger dykes†
SiO ₂	49.78±2.63	49.29±2.74
Al ₂ O ₃	12.77±0.44	12.69±0.40
TiO ₂	2.48±0.53	1.99±0.52
Fe as Fe ₂ O ₃	16.97±3.69	16.49±1.72
MnO	0.22±0.06	0.20±0.03
MgO	5.12±1.53	5.68±1.10
CaO	8.27±0.52	9.05±0.94
Na ₂ O	2.37±0.35	2.30±0.18
K ₂ O	0.91±0.07	0.84±0.20
P ₂ O ₅	0.44±0.10	0.27±0.11
H ₂ O-	0.10±0.05	0.23±0.15
L.O.I.‡	0.68±0.14	1.03±0.12
Rb (p.p.m.)	9.50	15.9
Sr (p.p.m.)	182.0	144.4
K/Rb	795.0	439.0
Rb/Sr	0.0521	0.110
C.I.P.W. norm§		
OZ	4.74	2.85
OR	5.38	4.96
PLAG	41.57	41.28
(% An)	(52)	(52)
CPX	13.87	17.71
OPX	21.77	21.49
AP	1.04	.64
MT	4.92	4.78
IL	4.71	3.78
Normative specific gravity	3.13	3.13

Major element concentrations were determined by X-ray fluorescence spectrometry. Trace element concentrations determined by isotope dilution mass spectrometry. The scatter in the analyses are expressed as single standard deviations.

*Average of 5 samples.

†Average of 7 samples.

‡Loss on ignition.

§Calculated assuming 80% of total Fe occurs as ferrous iron.

rocks were presumably deposited. Although no compelling field evidence for a sialic basement to the Barberton greenstone belt has yet been reported, petrogenetic considerations for the evolution of komatiitic magmas suggest that a continental environment existed³⁴. Furthermore, we and others (T. N. Clifford and C. Guerin, personal communication) have observed, on the south-western flank of the Barberton greenstone belt, outcrops of sialic gneisses containing strongly deformed basic dykes. These outcrops are

very similar in aspect to those of the Sand River area, and it now seems likely that detailed structural and isotopic studies, of the type reported in this paper, in the Barberton and Swaziland areas and in particular in the so-called migmatitic-gneiss complex terrains, will confirm the presence of ancient pre-greenstone belt sialic crust³⁵.

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Is an early calcium flux necessary to stimulate lymphocytes?

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Concentrations of concanavalin A or the calcium ionophore A23187 that are optimal for the transformation of pig or mouse lymphocytes do not normally cause a measurable increase in calcium influx compared with unstimulated cells. If the cells are treated with the mitogens in conditions where a measurable increase in calcium influx occurs, no stimulation of the cells can occur while the flux is maintained. If any early influx of extracellular calcium is necessary for stimulation, then a much smaller increase in the total concentration of cellular calcium than reported previously is sufficient to allow the entry of lymphocytes into the cell cycle.

SEVERAL reports have indicated that mitogens which stimulate the transformation of T lymphocytes cause an increased rate of calcium influx within 1 h (refs 1–6). The magnitude and duration of the calcium fluxes reported for both resting and stimulated lymphocytes, however, vary widely. Thus, Freedman *et al.*⁶ have described a gated calcium flux into mouse T cells stimulated by optimal concentrations of concanavalin (con A) in which a large influx is completed within one minute of the addition of the lectin, after which the gate shuts and the subsequent uptake of calcium is the same as in unstimulated cells. In contrast, Whitney and Sutherland³ have found that there was a very large and continuous increase in Ca²⁺ uptake

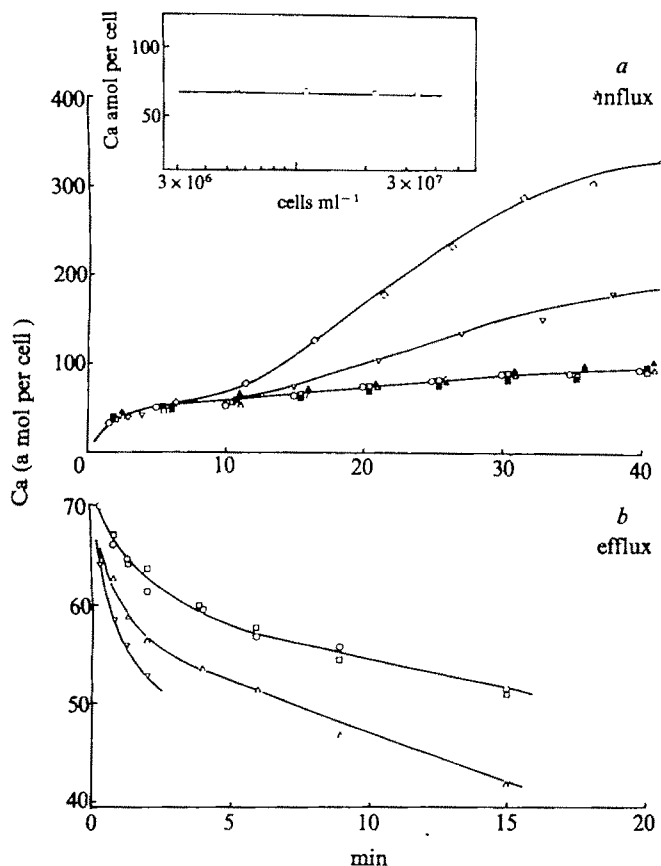


Fig. 1 a, Uptake of Ca^{2+} into unwashed pig lymphocytes (10^7 cells ml^{-1}) without FCS at 37°C, at increasing concentrations of ionophore. ○, Control; □, 0.076 μM ; △, 0.19 μM ; ▽, 0.95 μM ; ◇, 1.9 μM . Solid symbols indicate Ca^{2+} taken up by cells pre-incubated with ionophore for 1 h at 37°C before addition of $^{45}Ca^{2+}$. ■, 0.076 μM ; ▲, 0.19 μM . $^{45}CaCl_2$ (0.88 $\mu mol\ Ca^{2+}$ mCi^{-1} ; Amersham) in aqueous solution was added to culture medium to give a final activity of 6 $\mu Ci\ ml^{-1}$. This solution was filtered through a 0.22 μm Millipore filter to remove particulate matter, and aliquots were dispensed into cleaned plastic vials and incubated at 37°C. Filtering the incubation medium improved the reproducibility of calcium uptake levels between different cell preparations. A stock solution of A23187 (19 mM in DMSO) or con A (1 mg ml^{-1} ; Miles-Yeda) was diluted into filtered medium and added to the incubation followed by 50 μl of stock cell suspension to give a final concentration of 10^7 cells ml^{-1} in a total volume of 1.0 ml. Incubations were made under 5% CO_2 in air, with or without continuous stirring in each system¹⁷. Samples of 200 μl were withdrawn and diluted into 10 ml of wash buffer (either 146 mM NaCl, 10 mM Tris, 0.4 mM Ca-EGTA; or 146 mM NaCl, 10 mM phosphate, 0.4 mM Ca-EGTA, pH 7.2; or culture medium) at 3°C and immediately filtered through 2.5-cm Whatman GF/C filters. The cells were further washed with 30 ml of the same wash solution at 3°C within a total dilution and wash time of 4 s, and the filters dried and counted for radioactivity. A wide range of variations in this protocol were examined. The $^{45}Ca^{2+}$ retained was decreased by using larger wash volumes up to 60 ml (<10%); by lengthening the wash time to 45 s at 3°C (<2.5%); and by raising the temperature of the wash medium to 37°C (<45%), whereas removal of EGTA and the addition of 5 mM Ca^{2+} to the wash medium increased the $^{45}Ca^{2+}$ retained by up to 35%. The viability of the cells was unaffected by the filtration and washing procedures, as judged by their retention of $^{51}Cr^{3+}$ and their subsequent transformation by con A. We conclude that the calcium levels measured by the basic protocol represent most or all of the $^{45}Ca^{2+}$ that has been taken up or tightly bound to the cells during incubation at 37°C. In experiments in which transformation of cells from the same preparation was followed after the flux measurements, $^{45}Ca^{2+}$ uptake was measured at the optimal cell concentration for transformation of $3 \times 10^6\ ml^{-1}$. Aliquots of the cells from each incubation for $^{45}Ca^{2+}$ uptake were subsequently shown to be transformed to the same extent as control samples of the same cell preparation set up for transformation (see Fig. 2). **b**, Efflux of Ca^{2+} from unwashed pig lymphocytes loaded with $^{45}Ca^{2+}$ for 30 min at 37°C as for uptake measurements, and concentrated by centrifugation for 30 s at 14,000g (viability unaffected) before diluting into culture medium, with or without ionophore present, to give a final concentration of 10^7 cells ml^{-1} at 37°C. $^{45}Ca^{2+}$ retained in the preloaded cells was measured as for the uptake experiments. ○, Control; □, 0.19 μM ; △, 0.38 μM ; ▽, 1.15 μM .

for at least 24 h in cells stimulated with con A. Since the timing and magnitude of the early events after stimulation are critical in defining the triggering mechanism, and even a requirement for Ca^{2+} in the culture medium during the early stages of stimulation has been questioned⁷⁻¹⁰, we have attempted to determine whether either a gated pulse or a prolonged influx of calcium is obligatory for transformation. We have found that in conditions in which the mitogens cause a measurable increase in calcium influx in pig and mouse lymphocytes, the cells cannot become committed to transform while the influx is maintained. The limit to any calcium influx during the commitment of the cells to subsequent transformation corresponds to an increase in the total cellular concentration of less than 50 $\mu M\ Ca^{2+}$.

Calcium fluxes and transformation stimulated by A23187 and con A

Lymphocytes from pig mesenteric lymph node, or BALB/c mouse spleen were teased into culture medium containing RPMI 1640 (0.43 mM Ca^{2+}), supplemented with 24 mM sodium bicarbonate; 20 mM Tris-HCl; and gentamicin (10 $\mu g\ ml^{-1}$), pH 7.2. These unwashed cells were either used without further treatment, or the cells were washed by centrifugation on to 6.0% Ficoll and 16.7% Triosil, and the interfacial layer of cells centrifuged and resuspended twice in culture medium. For some Ca^{2+} uptake and transformation experiments (see Figs 1 and 2 for methods), 10% heat-inactivated foetal calf serum (FCS; Flow Labs) was also added to the culture medium. The FCS had little effect on DNA synthesis in transformed cells (for example compare Fig. 2a and b).

Calcium influx into unwashed pig lymphocytes in culture medium measured at 30 min in the absence of ionophore was found to be almost constant at 70–80 amol per cell, over the range of cell concentrations from 3×10^6 to 3×10^7 cells ml^{-1} (Fig. 1a, inset). This corresponds to an average cellular calcium concentration of about 0.6–0.7 mM, assuming a cell volume of 120 μm^3 (ref. 11), compared with an extracellular concentration of 0.43 mM. Calcium uptake into the cells at increasing concentrations of ionophore is shown in Fig. 1a. It can be seen that no significant stimulation of calcium uptake can be detected at ionophore concentrations less than 0.3 μM , but increasing the concentration further causes a progressive increase in $^{45}Ca^{2+}$ retained by the cells. Similar calcium uptake experiments were also performed with cells that had been pre-incubated for 1 h at 37°C with ionophore to ensure equilibration before adding $^{45}Ca^{2+}$. The uptake of $^{45}Ca^{2+}$ is unaltered (Fig. 1a), indicating that the absence of a detectable increase in calcium flux below 0.3 μM is not due to slow equilibration of the ionophore with the cells. Ionophore concentrations less than 0.3 μM that have no detectable effect on calcium uptake also have no effect on the efflux profiles from cells preloaded for 30 min with $^{45}Ca^{2+}$ without ionophore present (Fig. 1b). We can conclude that any change in the average cellular calcium concentration is too small to detect against the fluxes occurring in the control cells.

The transformation of the same cell preparations used to measure calcium uptake is shown in Fig. 2a. It is clear that a measurable increase in $^{45}Ca^{2+}$ uptake at 30 min only occurs at concentrations of ionophore that abolish transformation measured as DNA synthesis at 42–48 h. A very similar relationship between calcium uptake and transformation is observed in the presence of FCS (Fig. 2b). An increase in ionophore concentration of about tenfold is required for transformation, presumably because the serum binds a major portion of the ionophore. At the same time, the calcium uptake profile is shifted to higher ionophore concentrations, so that no increase in calcium uptake

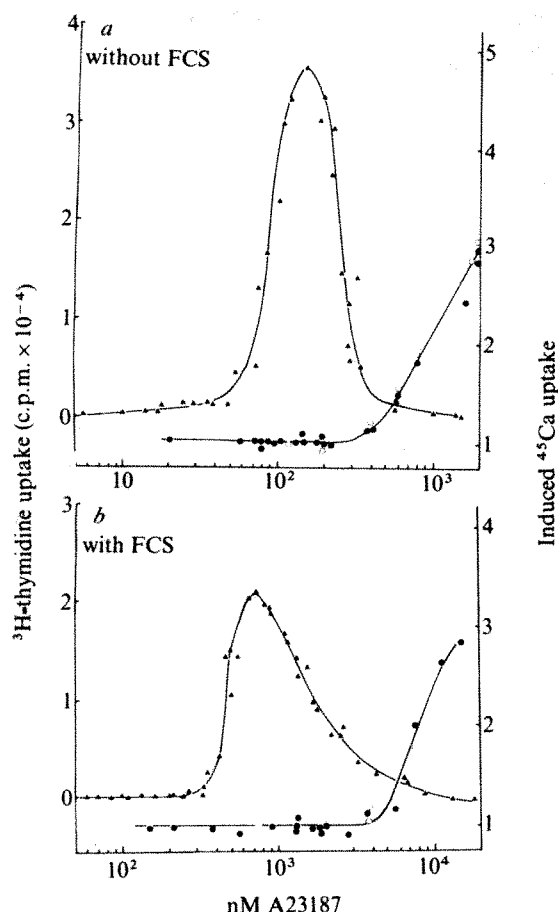


Fig. 2 *a*, Uptake of $^{45}\text{Ca}^{2+}$ into unwashed pig lymphocytes in culture medium without FCS after 30 min at 37°C as a function of ionophore concentration, expressed as a ratio to uptake in cells without ionophore. The uptake ratios were the same at 10^7 cells ml^{-1} ($\bullet$) and at 3×10^6 cells ml^{-1} ($\circ$), which was the optimal concentration for transformation measured as DNA synthesis ($\blacktriangle$) in the same cell preparation and medium. Aliquots (200 μl) of the cell suspension at 3×10^6 cells ml^{-1} were incubated with ionophore for 42 h in round-bottomed Cooke Microtitre plates under 5% CO_2 in air, before addition of 0.3 μCi ^3H -thymidine to each well. The cells were collected with a multiple harvester, washed with water and counted for radioactivity. *b*, Same data as in *a* with 10% FCS added to the medium.

is observed at ionophore concentrations that cause transformation.

In the transformation experiments with ionophore, the levels of DNA synthesis were very similar to those obtained with optimal concentrations of con A (Figs 2*a* and 3*a*). Removal of the ionophore after 24 h by centrifugation and washing the cells in fresh medium, approximately doubles the levels of DNA synthesis measured between 42 and 48 h. The highest proportion of pig cells responding to A23187 or con A was approximately 70%, as judged by a count of blast cells at 42 h, similar to the response to ionophore first described by Maino *et al.*¹²

The pattern of response to con A for calcium fluxes and transformation in pig and mouse lymphocytes is shown in Fig. 3*a* and *b*. For pig cells, no significant increase in calcium flux was observed at concentrations of con A between 0.1 and $100 \mu\text{g ml}^{-1}$. The average calcium uptake ratio at con A concentrations that cause significant transformation was 1.04 (s.d. of 0.04 in 26 experiments). The corresponding data for mouse cells shows a significant increase in calcium influx above about $5 \mu\text{g ml}^{-1}$ con A, where transformation is almost completely inhibited. Thus, although the calcium uptake data for con-A-stimulated cells is more variable than the corresponding ionophore

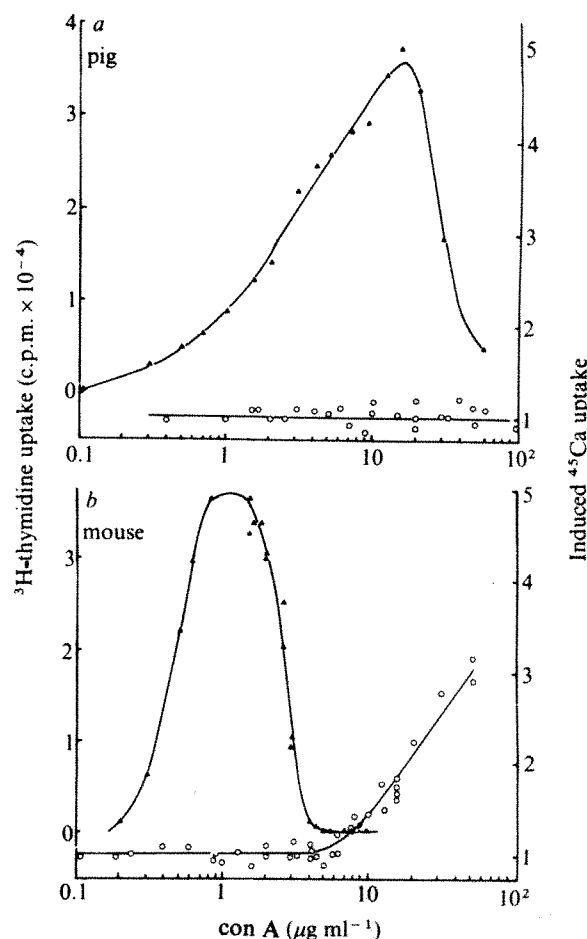
data, for both mitogens at optimal concentrations for transformation there is no significant increase in calcium influx at 30 min.

Commitment to transformation can occur after but not during an early calcium influx

The experiments described strongly suggest that an immediate calcium influx large enough to be measured against the influx into resting cells is not required for transformation, but is apparently inhibitory. We therefore attempted to induce large calcium influxes into the cells to determine whether they would remain viable, and in what conditions they would subsequently transform. Large increases in $^{45}\text{Ca}^{2+}$ uptake at 30 min were induced in pig cells by high concentrations of ionophore (Fig. 1*a*). If the ionophore was then removed by washing, and the cells resuspended in optimal concentrations of ionophore, the viability was unaltered and the cells subsequently transformed normally. Mouse lymphocytes were rapidly killed at ionophore concentrations optimal for pig cell transformation (40% viability after 3 h in $0.3 \mu\text{M}$ A23187) and only small stimulations of DNA synthesis corresponding to about 15% of the maximal stimulation of con A were observed.

In contrast to unwashed pig lymphocytes, the $^{45}\text{Ca}^{2+}$

Fig. 3 *a*, Effect of con A on the uptake of $^{45}\text{Ca}^{2+}$ into pig lymphocytes at 3×10^6 cells ml^{-1} ($\circ$) in culture medium without FCS after 30 min at 37°C expressed as a ratio to uptake cells without con A, and compared with DNA synthesis in the same cell preparation at 42–48 h ($\blacktriangle$). In experiments with con A, both $^{45}\text{Ca}^{2+}$ uptake and transformation were unaffected by washing the cells. *b*, Same data as in *a* for mouse lymphocytes.



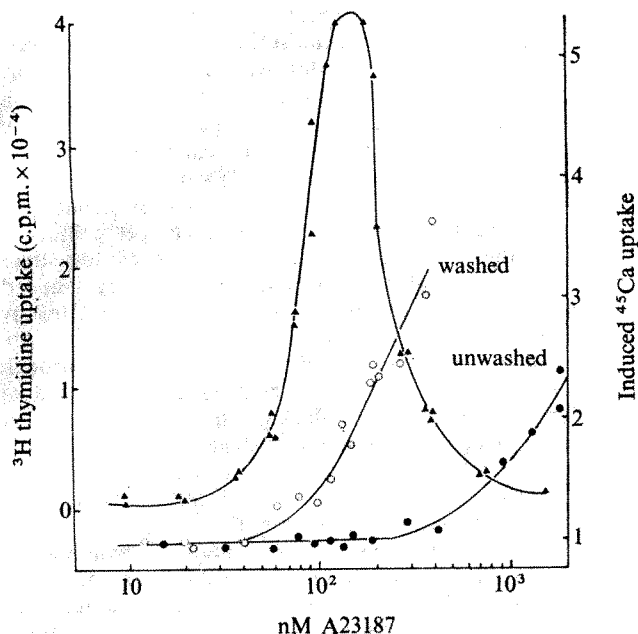


Fig. 4 Uptake of $^{45}\text{Ca}^{2+}$ into pig lymphocytes (3×10^6 cells ml^{-1}) either unwashed (●) or washed twice (○) (see text). DNA synthesis at 42–48 h (▲) in the same cell preparation was unaffected by washing the cells.

uptake profile was shifted to lower ionophore concentrations after washing the cells as described previously (Fig. 4). This treatment had no effect on the extent of transformation or the optimal concentration of ionophore required, so that the washed cells showed a significant increase in calcium uptake after 30 min at the ionophore concentrations optimal for transformation. When the time course of calcium uptake was followed by addition of $^{45}\text{Ca}^{2+}$ at increasing intervals after the addition of ionophore and comparing the uptake of $^{45}\text{Ca}^{2+}$ in cells with and without ionophore 30 min later, it was found that the initial increase in $^{45}\text{Ca}^{2+}$ uptake due to the ionophore decreased to the same level as in the control cells after about 4 h (Fig. 5). Subsequently the $^{45}\text{Ca}^{2+}$ uptake was indistinguishable from the control cells for at least 20 h, when the calcium uptake ratio was 1.02 (not shown). By analogy with the effect of FCS on the calcium uptake profile (Fig. 1a and b), it is likely that the large influx of $^{45}\text{Ca}^{2+}$ occurs when any extracellular material from the lymph tissue capable of buffering the ionophore has been depleted by washing, so that more of the ionophore interacts immediately with the cells than in the unwashed preparations. The subsequent decrease in $^{45}\text{Ca}^{2+}$ may result from redistribution, or even metabolism, of the ionophore in the cells. If the increase in $^{45}\text{Ca}^{2+}$ uptake is maintained by further additions of ionophore, no transformation occurs. On the other hand, if the optimal concentration of ionophore is added to the washed cells in ten equal aliquots at 30-min intervals, no significant increase in $^{45}\text{Ca}^{2+}$ uptake is observed, although the cells transform normally as if the ionophore had been added as a single dose.

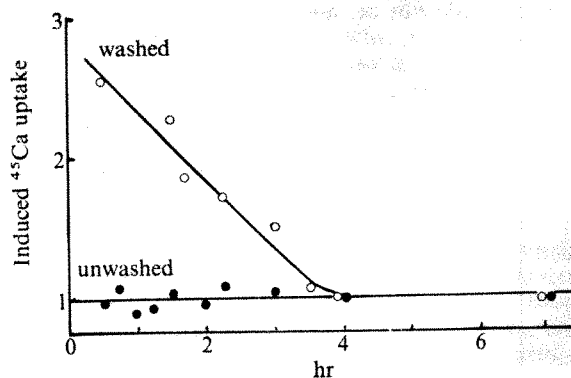
Calcium fluxes and transformation

Contrary to previous studies, the present results consistently demonstrate that a persistent increase in calcium influx large enough to be detected against the normal levels of calcium exchange in unstimulated lymphocytes is sufficient to abolish the subsequent transformation of the cells. We cannot account comprehensively for this discrepancy, although we would emphasise that to demonstrate the relationship between the calcium flux profile and

transformation (for example, in Figs 2, 3 and 4), it is essential that the measurements are carried out on a single cell preparation using the same mitogen solutions and at optimal cell concentrations for transformation. Small differences between cell preparations and larger differences between batches of mitogens were sufficient to completely obscure the pattern of results. This is particularly important in comparing calcium fluxes with transformations caused by short exposures (for example, 1–5 h) to high ionophore concentrations for which the optimal concentration is significantly higher than for long term stimulations (T.R.H. *et al.*, unpublished). The importance of the experimental conditions is also demonstrated by the facility with which apparently conflicting results can be generated by small changes in the treatment of the cells, where washing pig lymphocytes can induce a significant increase in calcium influx at optimal ionophore concentrations similar to the fluxes described previously. The stimulated influx, however, is transient, and the cells can only become committed to transform after the calcium influx has returned to the unstimulated level.

In most of the previous reports^{1–6,8–10} the increase in Ca^{2+} influx due to ionophore, con A or phytohaemagglutinin can be calculated as ranging between 60 amol per cell and 35×10^3 amol per cell, which if retained in the cell would correspond to an increase in total cellular calcium concentration of 0.5–300 mM. If these large fluxes occur in cells that are to transform, we assume that nearly all of the calcium would have to be pumped out of the cell again or be sequestered in organelles, since 1 mM calcium in the cytoplasm would be sufficient to inhibit glycolysis and protein synthesis, which are both stimulated in transformed cells^{13,14}. It is likely that at supraoptimal concentrations of ionophore, pig lymphocytes are unable to cope with the large and continuous calcium influxes from the medium (0.43 mM Ca^{2+}) and that the cytoplasmic concentration therefore increases to a level which prevents the generation of a mitogenic signal and is eventually cytotoxic. The only report in which the experiments carry the implication that the cell can successfully remove a large calcium influx from the cytoplasm, is the gated calcium pulse described by Freedman *et al.*^{6,9,10}. The kinetic implications of the pulse, which corresponded to an increase in the total cellular concentration of about 2 mM, are important in considering it as a possible mitogenic signal. When the con A and $^{45}\text{Ca}^{2+}$ were added to the mouse lymphocytes at the same time, the increase in the $^{45}\text{Ca}^{2+}$ uptake ratio remained constant after the first minute for at least 20 h (Freedman, M. H., personal communication),

Fig. 5 Uptake of $^{45}\text{Ca}^{2+}$ into unwashed (●) and twice-washed (○) pig lymphocytes, as a function of time. The optimal ionophore concentration for transformation (0.10 μM) was added at start, and $^{45}\text{Ca}^{2+}$ added after increasing time intervals. After 30 min incubation with $^{45}\text{Ca}^{2+}$, the uptake was measured and is expressed as a ratio to uptake in control cells.



whereas the uptake of $^{45}\text{Ca}^{2+}$ added 5 min after the con A, when the gate was shut, was the same as in control cells.

Systematic examination of kinetic models for the calcium fluxes after the gate has shut, in which we have assumed that the calcium entering the cell during the pulse remains accessible for subsequent exchange with the extracellular calcium, has indicated that all of the models require the $^{45}\text{Ca}^{2+}$ uptake ratio to drop from a maximum value after the gate shuts, towards the level of unstimulated cells. Since this is contrary to what has been observed experimentally, we conclude that the gated calcium pulse into the stimulated cells must be deposited immediately at intracellular sites where it is inaccessible for exchange. The subsequent exchange fluxes are then unaffected by the pulse and are the same as in unstimulated cells.

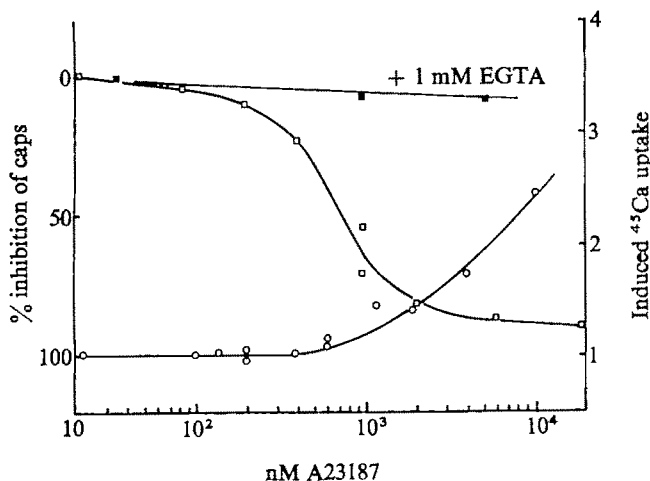


Fig. 6 Inhibition by A23187 of capping (± 1 mM EGTA $\blacksquare$, $\square$) by fluorescein-labelled con A (FITC-con A) on washed pig lymphocytes, compared with the calcium uptake ratio at 30 min for cells from the same preparation ($\circ$). Cells at $3 \times 10^6 \text{ ml}^{-1}$ were incubated in culture medium with ionophore for 20 min at 37°C ; FITC-con A ($200 \mu\text{g ml}^{-1}$) was added, and the cells fixed in 1% paraformaldehyde after a further 10 min at 37°C before examination by fluorescence microscopy. Caps, defined as fluorescent staining restricted to less than half of the cell surface, were present on $30 \pm 5\%$ of the cells, and the results are expressed as a percentage of the number of cells capping in the presence of ionophore. Similar inhibition of capping by the ionophore, was observed with fluorescein-labelled antibody to immunoglobulin which forms caps on more than 90% of the B cells in the absence of ionophore (not shown).

The important features of the gated pulse are that, although it is large, the calcium does not remain free in the cytoplasm where it would be accessible for exchange, and that after the gate shuts, the cell does not have to clear from the cytoplasm a continuous large influx of calcium. Thus the cytoplasmic functions essential for transformation would not be inhibited. Since the pulse is largest at optimal con A concentrations for transformation, is observed in T or B cells only when stimulated with the appropriate mitogens¹⁰, and is modulated by cyclic nucleotides⁶, it has many of the characteristics expected for a mitogenic signal. On the other hand, the absence of a detectable calcium pulse in the present work is unlikely to be due to any insensitivity in the technique used, because the calcium uptake levels in unstimulated cells are similar to those observed by the centrifugation methods, and reproducible increases in the calcium fluxes can be measured at supraoptimal ionophore and con A concentrations that show striking internal consistencies with the transformation data.

If we accept that in some experimental conditions the

cells can transform without requiring an increase in calcium influx that can be measured against the large exchange fluxes occurring in resting lymphocytes, we can set a limit to any increase in the total cell calcium concentration that is obligatory for transformation. For pig lymphocytes at optimal ionophore concentrations, the calcium influx ratio is 1.01 ± 0.06 (33 experiments with FCS) and 1.00 ± 0.08 (22 experiments without FCS). This is equivalent to a change in calcium flux of less than $\pm 6 \text{ amol calcium per cell}$, or a change in the total cellular concentration of less than $50 \mu\text{M}$ calcium. Similar conclusions apply to any induced calcium influx in pig or mouse lymphocytes stimulated by optimal con A concentration. These results raise the question of whether the ionophore or con A act through a calcium flux, and whether calcium is even required in the extracellular medium during the early stages of stimulation, which is at present unclear⁷⁻¹⁰.

There is a further complication in that there is compelling evidence that at least some T-cell subpopulations require macrophages for transformation¹⁴, and it has also been suggested that the ionophore stimulates lymphocytes indirectly by causing the release of soluble factors from macrophages. Although macrophages were not removed from the cell preparations used in the present studies, there is little doubt that the calcium fluxes at supraoptimal ionophore or con A concentrations were too large to be occurring in a small population of cells which were not lymphocytes. We therefore looked for evidence of a direct and immediate action of the ionophore on the plasma membrane of pig lymphocytes, and found that the ionophore inhibits cap formation by fluorescein-labelled con A, at concentrations at which an increased calcium flux can be detected (Fig. 6). This is similar to the observation by Schreiner and Unanue¹⁸ that the ionophore inhibits cap formation by antibody to the immunoglobulin receptor on mouse B lymphocytes, and strongly suggests that the ionophore is also interacting directly with the lymphocytes in the concentration range which causes transformation. Since the inhibition of cap formation requires calcium in the medium (Fig. 6), but not magnesium, we think it is very probable that the ionophore must cause an influx of calcium at optimal concentrations for transformation that is too small to measure by the present technique against the large fluxes in resting cells. The significance of such a small flux as a mitogenic signal and the requirement of extracellular calcium for the early stages of stimulation remain to be determined. The present work does not exclude the possibility that a small increase in cytoplasmic calcium concentration, within the limits described, is a component of the signal that commits the cells to transform.

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Endogenous opioid peptides: multiple agonists and receptors

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Opioid peptides were assayed by inhibition of ^3H -naloxone and ^3H -leu-enkephalin binding in brain homogenates and by depression of contractions of the guinea pig ileum and mouse vas deferens. We conclude that the opioid peptidergic system has agonists of different characteristics which interact with more than one type of receptor.

SOON after the publication¹ of the structures of Met-enkephalin and Leu-enkephalin and the fact that Met-enkephalin is present in β -lipotropin as the sequence 61–65, many papers have appeared which showed that peptides starting with the tyrosine residue 61 of β -lipotropin and having different lengths of amino acid residues interact with the opiate receptors in brain homogenates, guinea pig ileum and mouse vas deferens^{2–7}. The pharmacological properties of these peptides are sufficiently different that the question arises of whether or not they interact with identical receptor populations^{8,9}. Evidence for the presence of more than one species of opiate receptor may be of considerable importance in the analysis of the respective roles of the short- and long-chain opioid peptides. Related to this problem are recent findings which have made it doubtful that the many synthetic and semisynthetic surrogates of morphine in the morphine, morphinan, benzomorphan and phenylpiperidine series of compounds interact with a homogeneous group of receptors^{10–14}. Martin and his colleagues^{12,13} in particular have postulated from behavioural and neurophysiological observations on the chronic spinal dog that there are at least three different opiate receptors: typically morphine-like drugs interact with the μ -receptor whereas certain benzomorphans which do not substitute for morphine in the morphine-dependent monkey but are potent anti-nociceptive agents, for example ketocyclazocine and ethylketocyclazocine, interact with the κ -receptor. In addition, they distinguish the σ -receptor, for which the prototype is N-allylnorcyclazocine.

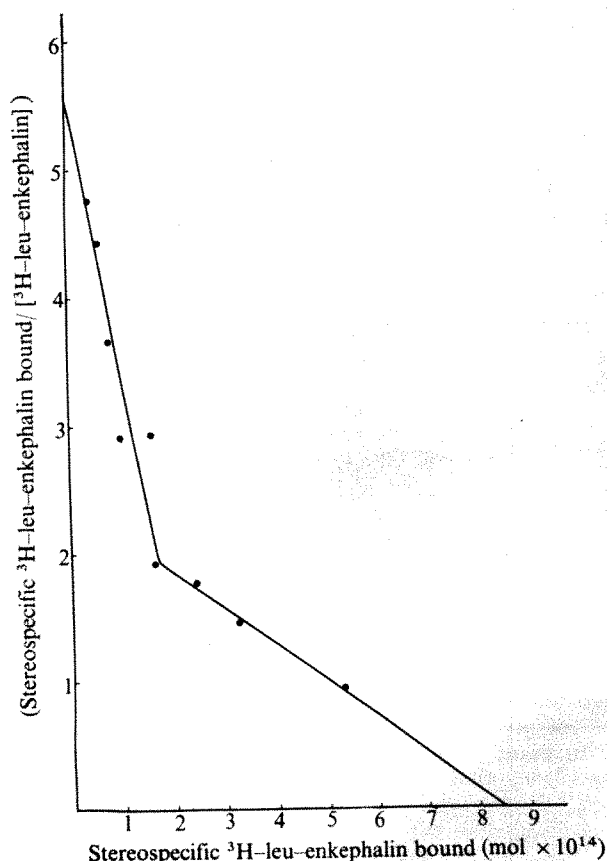
Differentiation of opioid receptors

Our aim was an analysis of this problem at the cellular level. Receptors of different binding properties present in a mixed receptor population are readily differentiated when antagonists are used which are specific for the different receptors. If such specific antagonists are not available, the agonist activities are assayed simultaneously in several systems. If the rank order of potency varies in parallel in the different assays, then the assay tissues may be assumed to have identical receptors. If, on the other hand, there is no correlation of the rank order of potency in the bioassay systems, the receptor populations are not identical¹⁵.

We used two assays based on pharmacological responses—the depression of the electrically-induced contractions of the guinea pig ileum or its myenteric plexus-longitudinal muscle preparation^{16,17} and of the mouse vas deferens¹⁸. The other two systems are based on the inhibition of binding of labelled ligands in brain homogenates^{19–21}. The primary ligands were ^3H -naloxone (4 Ci mmol^{-1} , 1 nM ;

National Institute on Drug Abuse) and ^3H -leu-enkephalin (45.6 Ci mmol^{-1} , 0.86 nM ; Radiochemical Centre, Amersham). The technique used has already been described⁸; the incubation of the homogenates of guinea pig brain (19 mg per 2 ml Tris buffer pH 7.4 at 0°C , without Na^+) was carried out at 0°C for 150 min to reduce enzymatic inactivation to its minimum. In these conditions maximum specific binding of either ligand was reached after 120 min. Specific binding was the difference between the values obtained in the absence and presence of 50 nM of the potent antagonist (–)2-(3-furylmethyl)-5,9-diethyl-2'-hydroxy-6,7-benzomorphan (Mr 2266 from Dr H. Merz, C. H. Boehringer)²². At the same concentration, the (+) isomer had no significant inhibitory effect on binding. The specific binding was 50–60% of the total binding. There are apparently two binding sites for ^3H -leu-enkephalin (Fig. 1), 18.8 $\pm$ 1.7% of which have a K_D of $0.47\pm0.09\text{ nM}$ and 81.2 $\pm$ 1.7% a K_D of $4.98\pm1.15\text{ nM}$ ($n=4$). Inhibition with cold Leu-enkephalin gave an ID_{50} (concentration for 50% inhibition) of $3.55\pm0.37\text{ nM}$ ($n=18$), a value close to that of the K_D of the binding site of lower affinity. The

Fig. 1 Specific binding of ^3H -leu-enkephalin in a homogenate of guinea pig brain, incubated at 0°C for 150 min. Abscissa, bound ^3H -leu-enkephalin (mol^{14} per 19 mg brain); ordinate, bound/free ^3H -leu-enkephalin.



ratio of binding at 0 and 100 mM Na⁺ is about 10 in our experimental conditions.

Correlation between responses of guinea pig ileum and mouse vas deferens

Investigations carried out over several years on a large number of narcotic analgesic drugs have established a good correlation between the relative agonist potencies in the guinea pig ileum and the mouse vas deferens, on the one hand, and their potencies for human analgesia, on the other^{18,23}. Recently, however, parallel assays of certain benzomorphans which are good anti-nociceptive agents but do not substitute for morphine in the morphine-dependent monkey, showed that these compounds can be distinguished by the fact that in the mouse vas deferens their relative agonist potency referred to normorphine as standard is consistently only 25% of that found in guinea pig ileum (Fig. 2)¹⁴. This finding suggests that these compounds, ketocyclazocines and *N*-dimethylfurylbenzomorphans, interact with a receptor which differs from the μ -receptor and is possibly identical with the κ -receptor of Martin and his colleagues^{12,13}. This view is strongly supported by the fact that in both preparations¹⁴ and in the chronic spinal dog²⁴ these compounds require three to six times more naloxone or naltrexone to reverse their agonist action than is needed for the antagonism of morphine and its surrogates which interact with the μ -receptor (compare with Table 2).

These observations on the heterogeneity of the opiate receptors are strongly reinforced by the behaviour of the endogenous opioid peptides. It is a striking feature that LPH₆₁₋₉₁ (C fragment or β -endorphin) has similar ID₅₀ values in the guinea pig ileum and the mouse vas deferens (Fig. 3). On the other hand, no correlation between the two *in vitro* assay models has been found for the shorter peptides, LPH₆₁₋₇₆ (α -endorphin), Met-enkephalin and Leu-enkephalin are progressively more potent in the mouse vas deferens than in the guinea pig ileum; in the latter preparation Met-enkephalin and β -endorphin are about equipotent but α -endorphin and Leu-enkephalin are less active. In contrast to these findings with the peptides, the mouse vas deferens is much less sensitive to the action of morphine than the guinea pig ileum. The possibility has to be considered that these discrepancies between the two *in vitro* preparations may be due to the fact that α - and β -endorphin are resistant to enzymic degradation whereas the enkephalins are rapidly inactivated by peptidases. It seems, however, that this possibility cannot be the main cause because, in the mouse vas deferens, α - and β -endorphin are the least active peptides and, in the guinea pig ileum, α -endorphin is less potent than Met-enkephalin.

From these observations we conclude that the receptor populations in the guinea pig ileum and mouse vas deferens

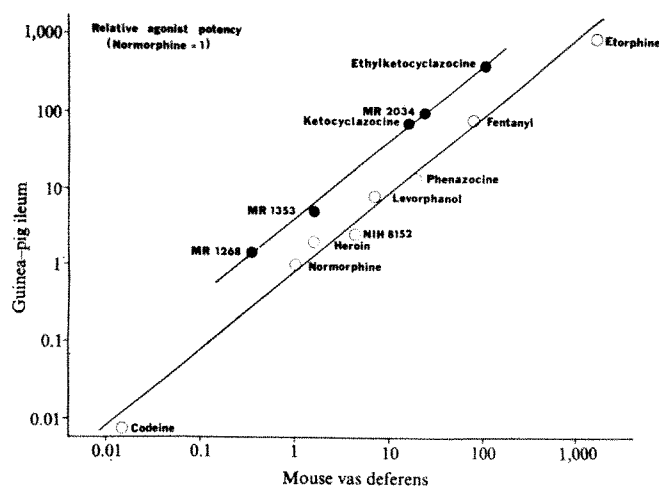


Fig. 2 Relative agonist activities of various narcotic analgesic agonists in the mouse vas deferens and guinea pig ileum (normorphine = 1). The compounds fitting the regression line on the right substitute and those fitting the line on the left do not substitute for morphine in the morphine-dependent monkey. Mr 2034, (–)- α -(1*R*,5*R*,9*R*)-5,9-dimethyl-2-(1-tetrahydrofurfuryl)-2'-hydroxy-6,7-benzomorphan (from Dr H. Merz); Mr 1353, (±)- α -5,9-dimethyl-2-(3-methylfurfuryl)-2'-hydroxy-6,7-benzomorphan; Mr 1268, (±)- α -5,9-dimethyl-2-(2-methyl-3-methylfuryl)-2'-hydroxy-6,7-benzomorphan; NIH 8152, (±)- β -2,9-dimethyl-5-*n*-propyl-2'-hydroxy-6,7-benzomorphan (from Dr E. L. May). (Courtesy of Frances M. Leslie).

are not homogeneous and identical. We now show that the opiate receptors present in homogenates of brain are also heterogeneous.

Inhibition of ³H-leu-enkephalin and ³H-naloxone binding in homogenates of guinea pig brain

When the inhibition of ³H-leu-enkephalin is compared to that of ³H-naloxone binding (Fig. 3), it is found that β -endorphin (LPH₆₁₋₉₁) is equipotent in these two systems. α -endorphin (LPH₆₁₋₇₆) is a weaker inhibitor of ³H-leu-enkephalin binding than β -endorphin; this reduction in potency is even more pronounced for the inhibition of ³H-naloxone binding. Met-enkephalin (LPH₆₁₋₆₅) is about equipotent with β -endorphin against ³H-leu-enkephalin binding but a weaker inhibitor of ³H-naloxone binding in confirmation of earlier observations². Leu-enkephalin is somewhat less potent than β -endorphin as an inhibitor of ³H-leu-enkephalin binding; a particularly interesting finding is its weak inhibition of ³H-naloxone binding against which it has an even lower potency than met-enkephalin. In contrast to the peptides, morphine is a much better inhibitor of

Table 1 Relative inhibitory potencies of Met-enkephalin analogues and β -endorphin on the contractions of guinea pig ileum and mouse vas deferens and on the binding of ³H-naloxone and ³H-leu-enkephalin by brain homogenates

Compound	Guinea pig ileum	Mouse vas deferens	Guinea pig ileum-mouse vas deferens	³ H-naloxone	³ H-leu-enkephalin	Naloxone-Leu-enkephalin
Met-enkephalin	1	1	1	1	1	1
Met-enkephalin amide	1.47 ± 0.18 (3)	0.71 ± 0.08 (3)*	2.1	0.61 ± 0.09 (4)*	0.33 ± 0.02 (3)†	1.8
N-CH ₃ -Met-enkephalin	1.45 ± 0.19 (6)*	0.23 ± 0.01 (5)†	6.3	0.53 ± 0.10 (3)*	0.29 ± 0.03 (4)†	1.8
N-CH ₃ -met-enkephalin amide	3.72 ± 0.96 (5)*	0.33 ± 0.04 (4)†	11	1.20 ± 0.10 (3)	0.08 ± 0.02 (3)†	15
D-Ala ² -met-enkephalin	6.01 ± 0.31 (8)†	5.60 ± 0.62 (7)†	1.1	0.54 ± 0.13 (3)*	1.67 ± 0.45 (3)	0.32

The values are the means ± s.e.; the number of observations are given in parentheses. The relative potencies were determined with Met-enkephalin as internal standard. Met-enkephalin amide and N-CH₃-met-enkephalin amide were provided by Drs A. F. Bradbury and D. G. Smyth. N-CH₃-met-enkephalin and D-Ala²-met-enkephalin were from Dr B. A. Morgan.

**P* < 0.05.

†*P* < 0.001.

^3H -naloxone binding than of ^3H -leu-enkephalin binding. The values obtained for naloxone are not included in Fig. 3 and show the same pattern as morphine, the ID_{50} value against ^3H -leu-enkephalin binding being $51 \pm 11 \text{ nM}$ ($n=5$) and against ^3H -naloxone binding $1.8 \pm 0.5 \text{ nM}$ ($n=3$).

Correlation between receptor affinity in the brain and pharmacological potency in the *in vitro* models

Although β -endorphin is equipotent as an inhibitor of ^3H -leu-enkephalin and ^3H -naloxone binding in guinea pig brain and is also equipotent in the guinea pig ileum and the mouse vas deferens, this relationship does not hold for the other naturally occurring opioid peptides. In guinea pig ileum, the potency pattern of the peptides is similar to that found for the inhibition of ^3H -naloxone binding; morphine, however, is more potent than may be expected from the inhibition of ^3H -naloxone binding. It has already been pointed out that, in the mouse vas deferens, α -endorphin, met-enkephalin and Leu-enkephalin are in this order progressively more active than β -endorphin, a pattern fundamentally different from that of inhibition of ^3H -naloxone binding; there is less discrepancy between the order of potency and the values found for the inhibition of ^3H -leu-enkephalin binding. Morphine is a weak inhibitor of ^3H -leu-enkephalin binding which agrees with its weak agonist activity in the mouse vas deferens. It seems, therefore, that in principle, the activity in the guinea pig ileum is better correlated to inhibition of ^3H -naloxone binding than to inhibition of ^3H -leu-enkephalin binding. In contrast, activity in the mouse vas deferens is not correlated to inhibition of ^3H -naloxone binding but seems to have a certain similarity to the pattern of the inhibition of ^3H -leu-enkephalin binding.

This relationship between the binding assays and the *in vitro* preparations is further illustrated when the binding and pharmacological properties of Met-enkephalin are changed by minor modifications of the molecule (Table 1). In the guinea pig ileum, amidation of the carboxyl group of the C-terminal methionine or the introduction of a methyl group in the amino group of the N-terminal tyrosine increases potency a little whereas in the mouse vas deferens activity is decreased, particularly of the latter analogue. The inhibition of ^3H -naloxone binding is about halved by both analogues and that of ^3H -leu-enkephalin reduced by about 70%. A combination of the two modifications yields $N\text{-CH}_3\text{-Tyr-Gly-Gly-Phe-Met-NH}_2$, with characteristics very different from its parent compound: it is almost four times more potent in the guinea pig ileum and only one-third as potent in the mouse vas deferens; it inhibits ^3H -naloxone binding as well as Met-enkephalin but has lost over 90% of its ability to inhibit ^3H -leu-enkephalin binding. Since the potency of Met-enkephalin is about eight times greater in

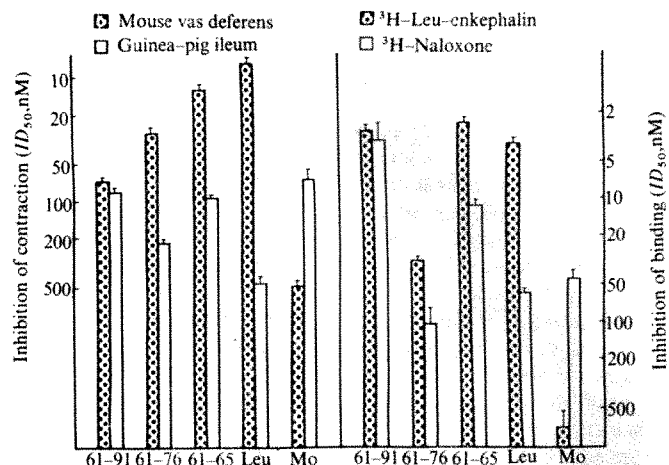


Fig. 3 The agonist activities of various compounds in the mouse vas deferens and guinea pig ileum and their potencies to inhibit ^3H -leu-enkephalin and ^3H -naloxone binding in homogenates of guinea pig brain ($\text{pH } 7.4$ at 0°C , no Na^+ , 150 min). The numbers on the abscissa indicate the amino acid sequence of β -lipotropin; Leu, leu-enkephalin and Mo, morphine. All peptides are synthetic (61-91, ovine β -endorphin, Dr C. H. Li; 61-76, porcine α -endorphin, Dr R. Guillemin; 61-65, met-enkephalin and leu-enkephalin, Dr B. A. Morgan).

the mouse vas deferens than in the guinea pig ileum, $N\text{-CH}_3\text{-met-enkephalin}$ amide is about 50% more potent in the guinea pig ileum than in the mouse vas deferens; further, since met-enkephalin inhibits ^3H -leu-enkephalin binding about five times more effectively than ^3H -naloxone binding, $N\text{-CH}_3\text{-met-enkephalin}$ amide is three times more potent against ^3H -naloxone than against ^3H -leu-enkephalin. A different picture is obtained when in met-enkephalin glycine² is replaced by the unnatural amino acid D-alanine. Compared with its parent compound, this analogue shows a sixfold increase in potency in both preparations, and is therefore about eight times more potent in the mouse vas deferens than in the guinea pig ileum. Its ability to inhibit ^3H -naloxone binding is halved but there is no reduction in its potency against ^3H -leu-enkephalin which therefore is inhibited about 15 times more than ^3H -naloxone. Thus, $\text{D-Ala}^2\text{-met-enkephalin}$ has a pattern of activity similar to that found for its parent compound, whereas $N\text{-CH}_3\text{-met-enkephalin}$ amide has in its relative, but not in its absolute, potencies acquired a pattern reminiscent of that of morphine. Both compounds show considerable resistance to inactivation by peptidases and after intraventricular or intracerebral administration exhibit antinociceptive activity^{25,26} which therefore does not seem to be closely and exclusively related to their affinity to receptors represented by either naloxone or leucine-enkephalin binding. $\text{D-Ala}^2\text{-met-enkephalin}$ is about equipotent with β -endorphin as an

Table 2 The effectiveness (K_e , nM) of naloxone and Mr 2266 to antagonise the agonist actions of normorphine, enkephalins and benzomorphans without physical dependence capacity

Compound	Naloxone*		Mr 2266†	
	Guinea pig ileum	Mouse vas deferens	Guinea pig ileum	Mouse vas deferens
Normorphine	2.64 ± 0.04 (3)	1.84 ± 0.20 (4)	1.93 ± 0.24 (4)	1.50 ± 0.14 (5)
Met-enkephalin	2.45 ± 0.26 (4)	22.6 ± 0.46 (4)	3.58 ± 0.08 (3)	16.2 ± 1.5 (4)
Leu-enkephalin	2.43 ± 0.67 (4)	21.4 ± 3.3 (4)	2.36 ± 0.44 (3)	34.0 ± 5.3 (6)
Normorphine	1.89 ± 0.15 (12)	3.10 ± 0.25 (6)	1.93 ± 0.24 (4)	1.50 ± 0.14 (5)
(±)-Ethylketocyclazocine	14.9 ± 0.9 (4)	11.0 ± 0.6 (3)	2.53 ± 0.60 (4)	4.51 ± 0.54 (6)
Mr 2034	10.4 ± 1.8 (7)	9.1 ± 0.7 (3)	3.34 ± 0.44 (3)	8.4 ± 1.3 (3)

Values are the means $\pm$ s.e.; the number of observations are given in parentheses.

*The values were obtained by a modification¹⁶ of the method of Arunlakshana and Schild²⁸ on segments of ileum. In the myenteric plexus-longitudinal muscle preparation K_e values were obtained by the 'single dose' method¹⁶: normorphine, $1.89 \pm 0.20 \text{ nM}$ (4), (±)-ethylketocyclazocine (Dr S. Archer) $11.9 \pm 1.2 \text{ nM}$ (3) and Mr 2034 $11.9 \pm 1.7 \text{ nM}$ (4).

†Mr 2266 (Dr H. Merz) has some agonist activity so the 'single-dose' method was used on the myenteric plexus-longitudinal muscle preparation and the mouse vas deferens.

antinociceptive agent in the rat²⁷ whereas *N*-CH₃-Met-enkephalin amide is less potent in the cat²⁸.

Differentiation of opioid receptors by action of antagonists

As explained previously, receptors of different binding properties present in a mixed receptor population are best differentiated by antagonists which are specific for the different receptors. The effectiveness of an antagonist against a given agonist is measured by the equilibrium dissociation constant K_e ; the value of this constant is equal to the antagonist concentration which requires a doubling of the agonist concentration to give the same pharmacological response as that obtained in the absence of any antagonist. K_e may also be expressed as its negative logarithm, pA_2 .

In the guinea pig ileum, the enkephalins are as readily antagonised by naloxone as is normorphine (Table 2). In mouse vas deferens, however, about 10 times more naloxone is required for the antagonism of the enkephalins than for that of normorphine. This low antagonist potency of naloxone in the mouse vas deferens seems to hold for all opioid peptides (Table 3), irrespective of whether they are more active in mouse vas deferens than in guinea pig ileum (for example, the enkephalins, α -endorphin and *D*-Ala²-Met-enkephalin), or not (β -endorphin and *N*-CH₃-Met-enkephalin amide).

These observations strongly reinforce the conclusions made from the pharmacological responses to the enkephalins and the endorphins, namely that their action in the mouse vas deferens is on receptors different from those on which morphine and its classical surrogates act. It is important to note that these receptors, which may be called δ -receptors⁸, are not responsible for the action of the enkephalins in guinea pig ileum. Since in this preparation the enkephalins are antagonised by naloxone as readily as morphine, they may be assumed to act on morphine- or μ -receptors.

As far as specific antagonists are concerned, no such antagonist has been found for the putative δ -receptor although the oripavine derivative diprenorphine is relatively more effective than naloxone⁹. The search for an antagonist acting on the putative κ -receptor has been somewhat more successful. In the guinea-pig ileum Mr 2266 is a potent antagonist of compounds acting on μ -receptors (for example, normorphine and the enkephalins) and also of the putative κ -agonists, ethylketocyclazocine and Mr 2034 (Table 2, see Fig. 2 legend for Mr 2034 structure). It seems from these experiments in the guinea pig ileum that, in contrast to naloxone, Mr 2266 acts equally well on μ - and κ -receptors. In the mouse vas deferens, however, Mr 2266

is not more effective as an κ -antagonist than naloxone. This difference between the two preparations is difficult to explain unless one assumes that there are only few κ -receptors in the mouse vas deferens whose relative insensitivity to putative κ -agonists has already been discussed.

Summary of evidence of opioid receptors

Investigation of the pharmacological responses of the guinea pig ileum and the mouse vas deferens to the inhibitory effects of the opioid peptides have led to the view that the receptor populations in the two *in vitro* models are heterogeneous and not identical. In the guinea pig ileum, the peptides interact mainly with the μ -receptor which mediates the action of the classical morphine-like compounds. In the mouse vas deferens, on the other hand, the opioid peptides interact mainly with the putative δ -receptors although they probably also affect the μ -receptors. It would seem likely that the δ -receptors are related to the binding sites in the brain for which ³H-leu-enkephalin has a high affinity while the μ -receptors may be related to the binding sites in the brain and the myenteric plexus of the guinea pig ileum which have a high affinity to ³H-naloxone^{19,29} and to ³H-dihydromorphine^{30,31}.

The guinea pig ileum also has receptors which are probably identical with the κ -receptors first postulated from the results of experiments on the chronic spinal dog^{12,13}; the mouse vas deferens seems to have fewer κ -receptors than the guinea pig ileum. It is not certain whether or not κ -receptors have a role in the action of the endogenous opioid peptides.

In view of the complexity of the opioid receptors, the use of a single assay system can no longer be considered a reliable method for prediction of the pharmacological activity of new opioid compounds, particularly of those with a peptide structure. For such compounds, multiple parallel assays similar to those used in the present investigation may be useful.

Implications of multiple receptors for physiological functions of opioid peptides

One of the most interesting findings has been the fact that β -endorphin inhibits equally well ³H-leu-enkephalin and ³H-naloxone binding, and is also equipotent in the guinea-pig ileum and mouse vas deferens. This observation may be interpreted as indicating that β -endorphin, which is possibly a precursor of other opioid peptides, interacts equally well with several or perhaps all opiate receptors. The shorter peptide, Met-enkephalin, is in several aspects different from its putative parent substance in that it has greater affinity to the binding site of ³H-leu-enkephalin than to that of ³H-naloxone, that it is more potent in the mouse vas deferens than in the guinea pig ileum and, finally, that it is readily inactivated by peptidases. Thus, it seems that Met-enkephalin may be designed for a more specialised function than β -endorphin.

The evidence available at present is insufficient to attempt to allocate different receptors to different physiological functions. We have already pointed out that antinociceptive action does not seem to be exclusively associated with any one receptor. It is, however, apparent that the opioid peptidergic system is of considerable complexity in that several agonists are matched by multiple receptors. These circumstances are similar to those found in the catecholamine system, but whereas in the latter system transmitter action is terminated by neuronal re-uptake, the opioid peptides are rapidly inactivated by enzymatic degradation, as is acetylcholine in the cholinergic system. Is it permissible to compare the neuroendocrine element of the catecholamine system, adrenaline, with the member of the peptidergic system, β -endorphin, which may also have a neuroendocrine function? Adrenaline is present in high

Table 3 Effectiveness of naloxone in antagonising the agonist actions of normorphine and opioid peptides in guinea pig ileum and mouse vas deferens

Agonist	Guinea pig ileum K_e (nM)	Mouse vas deferens K_e (nM)
Normorphine	1.89 ± 0.20 (4)	1.84 ± 0.20 (4)
Tyr-Gly-Gly-Phe	1.73, 1.77	29.2 ± 2.7 (4)
Met-enkephalin	2.45 ± 0.26 (4)	22.6 ± 0.5 (4)
α -Endorphin (61-76)	1.56 ± 0.15 (4)	22.5 ± 2.9 (4)
β -Endorphin (61-91)	2.86 ± 0.42 (5)	21.7 ± 1.2 (3)
<i>N</i> -CH ₃ -Met-enkephalin	2.07 ± 0.08 (4)	15.6 ± 3.8 (4)
<i>N</i> -CH ₃ -Met-enkephalin amide		16.6 ± 2.6 (4)
<i>D</i> -Ala ² -Met-enkephalin	2.55 ± 0.20 (4)	22.4 ± 2.7 (4)

K_e values were determined by a modification¹⁶ of the method of Arunlakshana and Schild²⁸ or the 'single dose' method¹⁶ and are the means ± s.e.; the number of observations are given in parentheses. Tyr-Gly-Gly-Phe was provided by Dr B. A. Morgan.

concentration in the adrenal medulla and β -endorphin and α -endorphin are found in the intermediate and anterior lobes of the pituitary gland, also in high concentrations³². Adrenaline is essential for the fight and flight reaction. β -Endorphin may be of similar importance: could the often reported lack of pain perception on the battlefield or the reduced pain experienced during childbirth be explained on this basis? In contrast, the other components of the two systems, noradrenaline and dopamine on the one hand, and Met-enkephalin and Leu-enkephalin on the other, are present in lower concentrations in widely-spread areas of the central and autonomic nervous systems^{33,34}. Immunohistochemical studies show that Met-enkephalin and Leu-enkephalin are widely distributed in neuronal fibres and terminals of the central nervous system of the rat^{35,36}. These findings and the rapid inactivation of the enkephalins are compatible with the role of neuromodulator or neurotransmitter suggested for them^{34,37}. If this concept is correct, it would follow that the enkephalins would subserve transmission of a rapid and more or less transient character while the longer-chain peptides, particularly β -endorphin would mediate neuronal and possible endocrine changes of a more lasting nature.

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letters to nature

Comments on the optical and radio detection of black hole explosions

It has recently been suggested¹ that it may be easier to detect the explosions of miniholes by the radio and optical pulses produced by the interaction of the emitted particles with the interstellar magnetic fields, than by the direct detection of the high energy γ rays².

We concern ourselves here first with the possibilities in the optical band. In the several attempts in recent years to detect fast transient phenomena in the visible band^{3–5}, including searches for X-ray bursts by the upper air atmospheric fluorescence technique^{6,7} the time constants have covered the region $\sim 100 \mu\text{s}$ to 10 s. Most of these experiments have been bedevilled by interference of one form or another, both man-made and natural.

The radiation mechanism proposed by Rees¹ is unique in that its timescale is far shorter than that for any other known phenomenon in the optical band. The pulse width or at least the rise time, for emission at a characteristic wavelength λ , on one of the models discussed, will be $\sim (\lambda/c)$ or $\sim 10^{-15}$ s. The shock front will be broadened by dispersion, both in the interstellar plasma and in the terrestrial atmosphere. The smearing due to the former, normally considered insignificant in the optical region, amounts to $\sim 5 \times 10^{-13}$ s over the band $\lambda = 400\text{--}600$ nm, if we accept a typical but arbitrarily chosen value of $DM = \int n_e dl \sim 50$ electrons cm^{-3} pc. The atmospheric dispersion is $\sim 2 \times 10^{-10}$ s for a scale height of 7 km and a

variance of 2.5% in $(n-1)$ over the same bandwidth. In either case, however, the timescales are much shorter than the response times of existing detectors, ~ 1 ns for small phototubes and ~ 10 ns for larger ones.

An optical search for shocks from minihole explosions has two practical advantages over radio. First, the fractional bandwidth which can be used is large, ~ 0.4 , and second, in the optical band, the only known sources of pulses on these timescales arise from Cerenkov flashes produced by extensive cosmic-ray showers⁸. This contrasts markedly with the situation at radio frequencies in which bandwidth-limited interference pulses are always observed, though in this case dispersion can be used to advantage for a celestial source.

If S_0 is the source strength in the optical band and we demand that the detected signal, on a timescale τ , shall exceed the threshold sensitivity set by the night-sky background noise, the distance d at which a single black hole explosion would be detected by a phototube of given quantum efficiency, with a mirror of effective area A and acceptance cone Ω , is

$$d = (S_0/4\pi)^{0.5} \text{const} (A^{0.25} \Omega^{-0.25}) \quad (1)$$

Since, however, the locations of the explosions are unknown, we must assume they are uniformly distributed and occur randomly over time. In this case, the observable volume will be

$$V = (\Omega d^3/3) = \text{const}' (A^{0.75} \Omega^{+0.25}) \quad (2)$$

From this we conclude that we require a flux collector

rather than a telescope, one with the largest collecting area and acceptance cone. The installations which have been used so extensively in high energy γ -ray astronomy⁹ therefore fulfil the requirements. In this technique, based on the Cerenkov effect of extensive cosmic-ray showers in the atmosphere⁸ resolving times τ of ~ 10 ns are in regular use and A and Ω are both large. For the large 10-m optical reflector at Mount Hopkins¹⁰, $A = 60 \text{ m}^2$ and $\Omega = 1.5 \times 10^{-4} \text{ sr}$ (ref. 7, p336). It is this flux collector which has already been used¹¹ to search for the γ -ray bursts originally predicted from minihole explosions².

The first example suggested in ref. 1, in which 10^{18} J might be emitted optically, in the case for $\gamma \sim 300$, gives an unfavourable prediction since the radiation from the fireball will be incoherent, and the duration of the pulse $\sim 0.1 \text{ s}$, just the time domain in which the interference problems are serious. It has, however, more recently been pointed out to us (M. J. Rees, personal communication) that the particle physics may be such that $E_{\text{opt}} \sim 10^{23} \text{ J}$, $\gamma \sim 10^7$ and hence that the radiation would then be coherent. In this case the prospects look much better. Taking the sensitivity of the 10-m reflector to be 5 photon m^{-2} (ref. 7, p.336) an explosion of 10^{23} J at $\lambda \sim 450 \text{ nm}$, for $\tau \sim 10 \text{ ns}$, would be detectable out to $d \sim 2 \text{ kpc}$ for which, from equation (2), $V \sim 4 \times 10^8 (\text{pc})^3$.

A multiplicity of spaced flux collectors would, however, be required, to suppress the chance-rate arising from Cerenkov flashes¹¹ from extensive cosmic-ray showers and other local phenomena. The intrinsic threshold detection rate for pbh explosions of this type would be $\sim 4.10^{-5} \text{ yr}^{-1} (\text{pc})^{-3}$ for the above volume and an observing time of $\sim 500 \text{ h}$. To achieve such a limit however, T. C. Weekes (personal communication) has pointed out that about five stations would be required, for an air-shower rate $\sim 10 \text{ s}^{-1}$ at each station and a coincidence resolving time $\sim 1 \text{ ms}$.

Turning now to the question of detecting radio bursts from pbh explosions, the situation is different, in that certain limits can already be set from experiments in other areas. In the radio region, the relationship between A and Ω is fixed, by the coherence properties of antennas.

If the energy emitted by a minihole explosion in unit bandwidth in the radio region is S_R , then the maximum distance for the detection of such an explosion is given by $d_{\text{max}} = (S_R/4\pi\epsilon_R)^{0.5}$ where ϵ_R is the energy threshold per unit area per unit bandwidth of an actual receiver system. For a single radio receiving system working at a specified frequency, $A\Omega$ is a constant, and $\epsilon_R \propto A^{-1}$, so that in the radio case, the corresponding expression to (2) is

$$V = \text{const}'' A^{0.5} \quad (3)$$

The achievable limits on the number of miniholes producing radio pulses is then proportional to $(A^{0.5}T)^{-1}$ where T is the total observing time of the experiment. The many radio experiments quoted in the literature can be divided broadly in two classes; firstly those involving small wide field of view antennas at low frequencies run for long periods of time, and secondly, those involving larger directional antennas at higher frequencies usually run for shorter periods. The duration of the emitted pulse is not a dominant consideration in most of the experiments as, provided the pulse duration, to include broadening by dispersion, is less than the system integration time, it is only the total energy in the pulse that determines the threshold level.

The characteristic frequency ν_c for the radio emission estimated by Rees, varies as a high power of γ_f ($\sim 2 \times 10^{16} \text{ g } M_{\text{crit}}^{-1}$); for $\gamma = 10^5$, $\nu_c \sim 3 \text{ GHz}$. Without knowing the values of γ , the optimum frequency cannot be selected in advance. We have calculated therefore the upper limits on the rates of such explosions, R , (99.9% confidence level) for two representative systems; one in each of the above classes (Table 1). In comparing these limits with those obtained in the γ -ray searches¹¹ it must be borne in mind that the radio and optical emission predicted by Rees is critically sensitive to

Table 1 Upper limits on the rates of explosions for two representative systems

System	ν (MHz)	$\delta\nu$ (MHz)	Antenna	Ω (sr)	Duration of experiment (h)	R ($\text{pc}^{-3}\text{yr}^{-1}$)
Small wide field of view antennas run for long periods ¹²	151	1	Dipoles	~ 1.3	3620	7×10^{-4}
Larger directional antenna run at a higher frequency for shorter periods ¹³	1665	15	25.6m dish	2.2×10^{-4}	28	0.1

the value of γ_f , which depends on the particle physics assumed.

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Optical pulses from primordial black hole explosions

REES¹ has pointed out recently that explosions of small black holes² could be detected by the radio or optical emission from an expanding relativistic shock wave interacting with the ambient magnetic field. The frequency of the radiation is determined by the value of a parameter $\gamma_f = (2 \times 10^{16} \text{ g}/M_{\text{crit}})$, where M_{crit} is the mass at which the black hole explodes. Jelley *et al.*³ have considered the optical emission and the radio emission which will occur for $\gamma_f \sim 10^5$, and shown that upper limits can be set for the rate of explosions, from previous measurements at 150 and 1,700 MHz.

For a lower value of γ_f , corresponding to the nuclear model of Hagedorn⁴, optical emission will be produced, but it will be incoherent, and the fraction of the total energy appearing is small. A limit has been set for γ rays for this model, using the atmospheric Cerenkov technique, by Porter and Weekes⁵. As Jelley *et al.* have pointed out, Cerenkov systems are suitable also for detecting fast optical pulses. For a γ_f value of 10^7 , 10^{30} erg of optical radiation

are predicted by ref. 1. This would correspond to a nuclear model at the opposite extreme from the Hagedorn model, and it is not clear whether the black hole explosion could occur in a sufficiently short time to give the required shock front.

If such an optical pulse is produced, however, the measurements made in ref. 5 can be used to provide an upper limit for the explosion rate. The optical sensitivity of the detectors was $370 \text{ photons m}^{-2}$ corresponding to $1.6 \times 10^{-13} \text{ erg cm}^{-2}$. The maximum distance for detection is then $r = E^{1/2}/2\pi^{1/2}F^{1/2}$, where E is the total energy produced and F the optical sensitivity. With $E = 10^{30} \text{ erg}$, r is $7 \times 10^{20} \text{ cm}$, or 230 pc. With the solid angle of $4 \times 10^{-3} \text{ sr}$ available, this gives a sensitive volume of $1.5 \times 10^4 \text{ pc}^3$.

The observed one event in 15.3 h of observation³, which is compatible with random expectation, corresponds to an upper limit of 6.8 events at the 99% confidence level. This yields an upper limit of $3.9 \times 10^3 \text{ events yr}^{-1}$ and hence an upper limit for the explosion rate of $0.3 \text{ events pc}^{-3} \text{ yr}^{-1}$. The limit found for γ rays was of the same order, but this was, of course, for the Hagedorn or Composite Particle model, in which 10^{30} erg are released at the explosion.

The sensitivity of this experiment could be improved by the use of a larger array of separated detectors, to reduce the chance rate, and a longer running time. In order to reach the sensitivity quoted by Jelley *et al.*, however, at least four very large optical reflectors each of aperture greater than 10 m and separated by more than 10 km, would be required. This calculation assumes a coincidence resolving time of 10^{-4} s and a running time of 500 h.

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Neutrino angular momentum losses in stellar collapse

WE know from observation that even the most rapidly rotating neutron stars—those of the Crab and Vela—are slow rotators, in the sense that their angular momentum is of negligible structural importance. In fact, at least for these pulsars, estimates based on their current slowdown rates indicate their rotation frequencies at birth were probably only a few times higher than the present ones¹, even though the main sequence progenitors probably had much more angular momentum per unit mass. The purpose of the present note is to examine the degree to which one can attribute such a decrease in the angular momentum per unit mass to neutrino radiation emitted during the formation of the neutron star from the collapse of a sufficiently massive stellar core.

According to the current theory, pulsars are presumably neutron stars which originate from the collapse of the cores of sufficiently massive stars ($M \geq 7M_{\odot}$) (ref. 2). After thermo-

nuclear burning has stopped, such stellar cores reach a state of very high density and temperature, so that the electron chemical potential is high enough to favour electron absorption on to protons ($e^- + p \rightarrow n + \nu_e$) over the reverse reaction (β decay). Electrons are thus eliminated, and so their contribution to the pressure decreases, causing the core to collapse. At the same time, vast amounts of neutrinos are produced by the inverse β decay and other thermal processes, taking up all the gain in gravitational energy arising from the collapse. The core finally settles down at densities of the order of $10^{14} - 10^{15} \text{ g cm}^{-3}$, it is then supported by nuclear degeneracy. One expects the stellar core to be rotating, increasing its angular velocity while collapsing by conserving angular momentum. The neutrinos leaving the core are emitted from a surface shell, approximately one mean free path thick. These neutrinos were initially produced throughout the core and not necessarily near its surface. If the rotation of the core is uniform, neutrinos of low angular momentum regions interact before escaping with the high angular momentum material of the surface. The result of such an interaction will be the neutrinos will gain angular momentum and so the angular momentum per unit mass of the escaping core will fall.

Let us consider a simple model, which allows analytic calculations and includes the general features of the actual situation, in order to estimate the effect neutrinos can have on the rotation of such a collapsing core. Consider a homogeneous spherically symmetric and uniformly rotating core, collapsing from density $\sim 10^{12} \text{ g cm}^{-3}$ to $10^{15} \text{ g cm}^{-3}$, and neutrinos take away all the gain in potential energy arising from the collapse.

In this core consider a spherical shell between r and $r+l$ (Fig. 1) ($r \gg l$, $l \approx$ neutrino mean free path). Let $E(r)$ be the

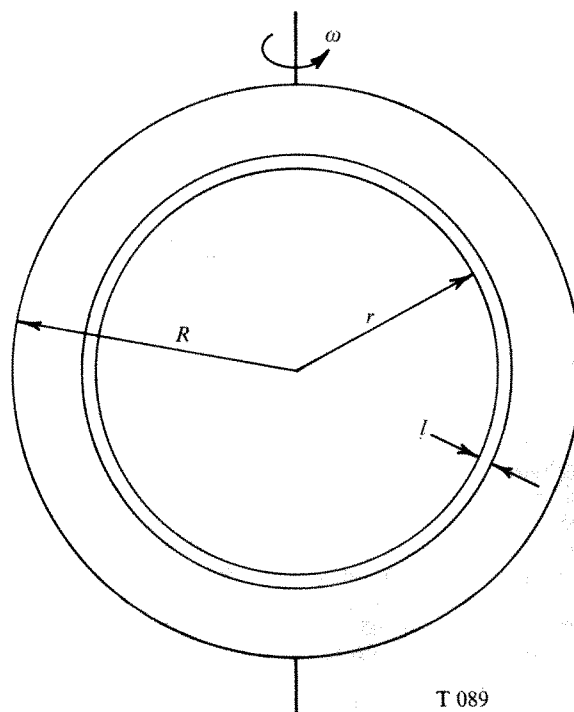


Fig. 1 Section through a burnt-out stellar core.

rate at which energy in the form of neutrinos is leaving the mass inside radius r . These neutrinos are emitted isotropically in the fluid's rest frame. Owing to the relativistic 'head light effect'³ from an inertial frame emission will seem to take place preferentially along the direction of rotation, resulting in a net angular momentum loss. This loss is further enhanced by a Doppler shift in the energy of the forward-against the backward-

emitted neutrinos. It is then easy to show that the part of the core inside radius r is losing angular momentum at a rate

$$\dot{J}(r) = -\frac{7}{9} \frac{\dot{E}(r)}{c^2} \omega r^2 \quad (1)$$

where ω is the angular velocity of rotation. These neutrinos and the corresponding angular momentum, are of course deposited in the shell between r and $r+l$, and then re-emitted at radius $r+l$, taking away angular momentum at a rate $\dot{J}(r+l)$. The net effect on the shell, therefore, will be to lose angular momentum at a rate

$$\dot{J}(r) - \dot{J}(r+l) = -\frac{7}{9} \frac{\dot{E}(r)}{c^2} 2\omega r l + O\left(\frac{l}{r}\right)^2 \quad (2)$$

Summing up the angular momentum loss rate over all shells, the total angular momentum loss rate is found to be

$$\dot{J}(R) = -\frac{1}{3} \frac{7}{9} \frac{\dot{E}(R)}{c^2} \omega R^2 \quad (3)$$

where R is the core radius and $\dot{E}(R)$ the total energy loss rate, which for the homogeneous sphere assumed in our case is

$$\dot{E}(R) = -\frac{3GM^2}{5R^2} \dot{R}$$

where M is the mass of the core.

The evolution now of angular velocity ω with R as the core collapses is easy to be followed by the equation

$$I\dot{\omega} + \dot{I}\omega = \dot{J} \quad (4)$$

where $I = 2/5 MR^2$, is the moment of inertia of the collapsing core. Straightforward integration of equation (4) gives

$$\omega = \omega_0 \left(\frac{R_0}{R}\right)^2 \exp\left[-\frac{1}{2} \frac{7}{9} \frac{GM}{c^2 R_0} \left(\frac{R_0}{R} - 1\right)\right] \quad (5)$$

(The subscript zero denotes the initial values of the corresponding quantities.)

The loss of neutrino angular momentum affects only the value of the exponential factor in equation (5) (see Fig. 2). Since the mass of the collapsing core and the ratio of its initial to final radius determine the total energy available to neutrinos for slowdown, it is not surprising that the neutrino angular momentum depends on them.

If we substitute realistic values for these quantities we can estimate how big the effect should be. For $R_0/R = 10$ and $M = 1.5M_\odot$ we find the value of the exponential factor to be 0.91. If we take $M = 2.5M_\odot$, which is close to the highest non-rotating neutron star mass allowed by an asymptotic free quark equation of state (Kislinger & Morley, personal communication), then its value is reduced to 0.88. So neutrinos seem to decrease ω from its expected value $\omega_0 (R_0/R)^2$ by 9% and 12% respectively for the two masses considered here. At even higher masses cores collapse to black holes. The magnitude of the effect then reaches a maximum ($\sim 18\%$ decrease), independent of the mass of the collapsing core, corresponding to $R = r_s = 2GM/c^2$ (the Schwarzschild radius) and $R_0 \rightarrow \infty$.

We have shown that neutrinos tend to slow down the rotation of a collapsing stellar core by carrying away part of its angular momentum. The effect seems to be present in all rotating collapsing cores, but does not completely account for the slow rotation of the compact supernova remnants (pulsars). But the angular momentum taken away by neutrinos and deposited in

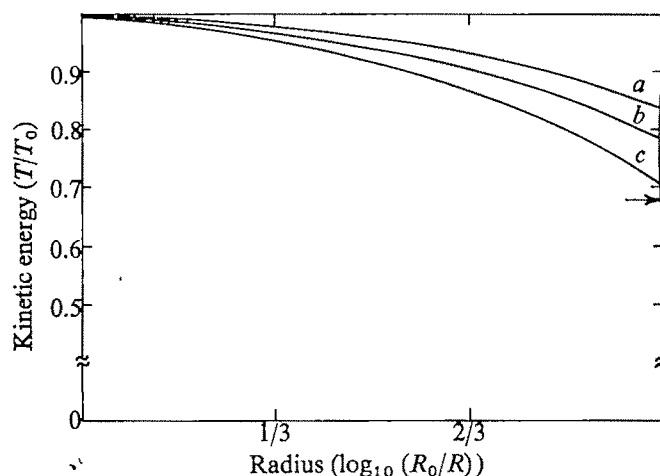


Fig. 2 The kinetic energy of the collapsing core T , measured at any instant in units of its value if no angular momentum losses are present T_0 , as a function of the core radius. *a*, For $M = 1.5M_\odot$ and $R/R_0 = 0.1$; *b* for $M = 2.5M_\odot$ and $R/R_0 = 0.10$; *c*, collapse to a black hole with $r_s/R_0 = 0.1$. The arrow indicates the (minimum) value of the ratio T/T_0 in the case that $r_s/R_0 \rightarrow 0$.

the expanding envelope may have some observational effects (that is, axial rather than spherical symmetry of the ejecta) or may help the explosion. Generalisation also of the above spherically symmetric treatment to sufficiently slowly rotating McLaurin spheroids gives essentially the same results. If we do not assume that rotation is uniform, the slowdown effect is smaller. In such a case neutrinos will act as sort of radiative viscosity smoothing out inequalities in the angular velocity.

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Small scale auroral absorption events associated with substorms

We describe the first results obtained from a new facility designed to study auroral absorption events exhibiting small scale spatial structure and rapid temporal variations. The absorption is measured by means of a modern solid-state riometer with high time resolution¹ and the spatial resolution is obtained using a narrow-beam antenna with 64 elements. The most striking observations made so far involve intense, short-lived 'spikes' of absorption associated with the onsets of auroral substorms. These spikes, which seem to be of small spatial scale, are probably related to the sudden ionospheric absorption (SIA or SAI) events reported by previous workers²⁻⁵ and perhaps to the 'spikes' of intense energetic electron precipitation observed from polar-orbiting satellites⁶⁻¹¹. Five such spikes were observed during November and December 1975.

The antenna array is constructed of elements made of coaxial cable (RG-62) with the inner and outer conductors interchanged at half-wavelength intervals¹². Eight such elements, each equivalent to eight half-wave dipoles, are mounted with a half wavelength between adjacent elements and a quarter wavelength above a ground plane. The antenna was first set up in Lindau and its radiation pattern determined by flying a portable transmitter through it. The predicted and observed patterns are in reasonably good agreement with $\sim 16^\circ$ between half-power points and with the side-lobes suppressed about 10 dB below the main beam (see Fig. 1). A similar array was erected in Ramfjord ($L \sim 6.2$) near Tromsø in northern Norway at the end of 1975. The riometer, which operates at a frequency of 51.4 MHz, has a band width of 150 kHz and a time constant of 0.25 s for both increasing and decreasing signal intensities¹.

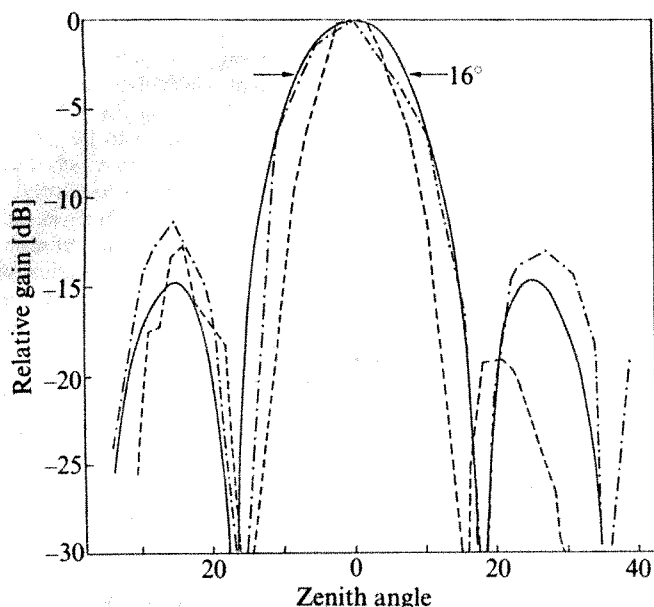


Fig. 1 Power polar diagram of the riometer antenna. The full-drawn curve represents the calculated pattern, while the two other curves are results of measurements of the directional variation of the antenna gain. The transmitting antenna used in these measurements was a crossed Herizian dipole taped on to the underside of the non-metallic body of an aircraft.

An example of one of the more impressive spikes observed during the first few months of operation of the system is shown in Fig. 2a. The absorption is shown as the equivalent absorption at 30 MHz assuming that the absorption varies inversely as the square of the frequency. The absorption observed simultaneously from a broad-beam 30-MHz riometer is also shown for comparison. The spike has a duration of approximately 1 min and the maximum absorption at 51.4 MHz is ~ 8 dB, corresponding to ~ 25 dB at 30 MHz. The maximum absorption measured by the broad-beam 30-MHz system was significantly smaller (~ 7.5 dB), which suggests that at the time the absorbing region did not completely fill the beam. Following the spike, there is a good correspondence between the two sets of observations. This indicates that the region of electron density enhancement in the D layer which produced the absorption had a spatial scale comparable with, or larger than, the field of view of the antennas and that the bulk of the absorption occurs at an altitude where the signal frequency is much larger than the electron-neutral collision frequency (that is, $\gtrsim 60$ km).

The absorption spike, which occurred at ~ 1750 UT on 3 November 1975, is coincident with the very sharp onset of a negative bay ($\sim 680\gamma$, Fig. 2b) and a rapid declination variation (~ 150 arc s), both of which had time scales

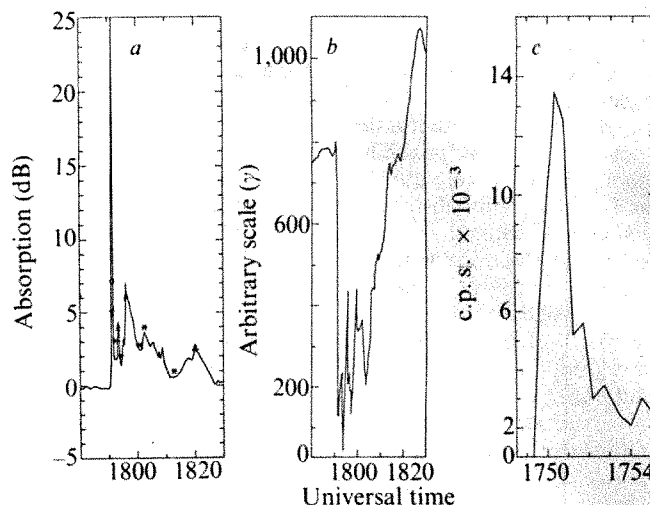


Fig. 2 a, An example of a very intense spike in ionospheric absorption occurring at Ramfjord at ~ 1750 UT on 3 November 1975. The circle and the stars indicate absorption measured using a broad-beam 30-MHz antenna (P. Stauning, personal communication). The absorption measured directly is marked with a circle, while absorption measurements corrected for zenith angle effects are marked with a star. This correction is made with the assumption that the sky temperature and absorption are homogeneous. b, Storm magnetogram from Tromsø showing H component of magnetic field (A. Brekke, personal communication). The absorption event occurred during the negative bay. c, ATS-6 36-keV electron measurements (C. E. McIlwain, personal communication). The counting rate started to increase about 1 min before the absorption, which may indicate that the magnetic conjugate point to ATS-6 was located south of Ramfjord before the absorption event.

of the order of 1 min. The substorm onset was observed simultaneously by energetic particle detectors on the geostationary Earth satellite ATS-6, which at the time has its geomagnetic conjugate point located 0.9° east and 1.0° south of our ground station in Ramfjord¹³. As observed at the satellite, the substorm was characterised by an increase by a factor of ~ 200 in the intensity of 36 keV electrons which took place with a timescale of the order of 1 min at 1750 UT. The electron intensity was observed to decay by a factor of about 10 during the next few minutes, so that in effect an electron spike was observed with a time duration of the order of 1 min. The 36-keV electron count rate is shown on a linear scale in Fig. 2c for comparison with the absorption measurements.

It is not completely clear how these observations can be interpreted in the absence of other evidence. One possibility, however, is that an absorbing region, spatially restricted in one direction but extended in the other, passed rapidly across the point of observation. If we assume that the vertical pencil beam absorption has the form $A(x) = A_0 \exp(-x^2/2x_0^2)$, where x is the horizontal distance from the line of maximum absorption, then, using both narrow- and broad-beam absorption measurements, one finds that the width of the absorbing region ($2x_0$) must have been about 50 km or 0.5 degrees of latitude if the absorption is assumed to occur at an altitude of ~ 80 km. Simultaneous broad-beam observations at 30 MHz and 40 MHz are consistent with this interpretation (P. Stauning, personal communication). The spatial scale we have deduced is consistent with the latitudinal width of energetic particle spikes observed by low-altitude satellites⁸⁻¹¹. Since the absorbing region must pass through the riometer antenna beam and intersect the conjugate point to ATS-6 within a time span of ~ 1 min at the most, the horizontal speed of the absorbing region must be quite considerable (that is, ~ 1 – 10 km

s^{-1}). This speed is similar to that of the poleward surge of auroral substorms¹⁴. Unfortunately we are unable to determine from the available all-sky camera records (one picture every 5 min) whether or not an arc-like structure passed rapidly over our observation point at the time in question.

An alternative possibility is that the effects we have observed are largely due to variations in the energy spectra and pitch-angle distributions of precipitating electrons associated with the substorm. Indeed, intense spikes of energetic electrons with pitch-angles almost parallel to the local magnetic field direction have recently been observed by the polar orbiting satellite OGO-6 (ref. 16). If, however, the bulk of the electrons involved have energies which are not greatly in excess of ~ 400 keV it is difficult to explain the difference between the relatively intense absorption observed at 51.4 MHz in comparison with that at 30 MHz. Of course some combination of these suggestions may be required to give a fully satisfactory explanation of the observations.

The absorption spikes have so far only been observed during the evening hours (1900–2200 CMLT). This result is consistent with statistical studies of riometer absorption¹⁵, which reveal one maximum of occurrence in the evening hours and with the pattern of occurrence of particle spikes observed by satellites¹¹. Auroral observations made from ISIS-2 also suggest that the initial brightening of substorms occurs more frequently in the late evening sector than in the midnight sector¹⁴.

We plan to extend the scope of these observations by making suitable changes in the system. A second broad-beam 51.4-MHz system is being set up in order that a definitive distinction can be made between spectral and spatial effects. We also intend to make photometer and television observations to see whether or not similar phenomena occur in the optical emissions. Simultaneous observations will be made using the VHF radar system 'STARE' which was recently put into operation¹⁷, and correlations will be made with energetic particle observations from the satellite GEOS.

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A proposed cadmium-selenium film-electrolyte Schottky barrier solar cell

ECONOMIC analyses of photovoltaic solar cells show that a considerable manufacturing cost reduction will be required before such devices can give a positive contribution to the World's energy needs. To this end, efforts have been made to divert some research work from the high-quality single-crystal

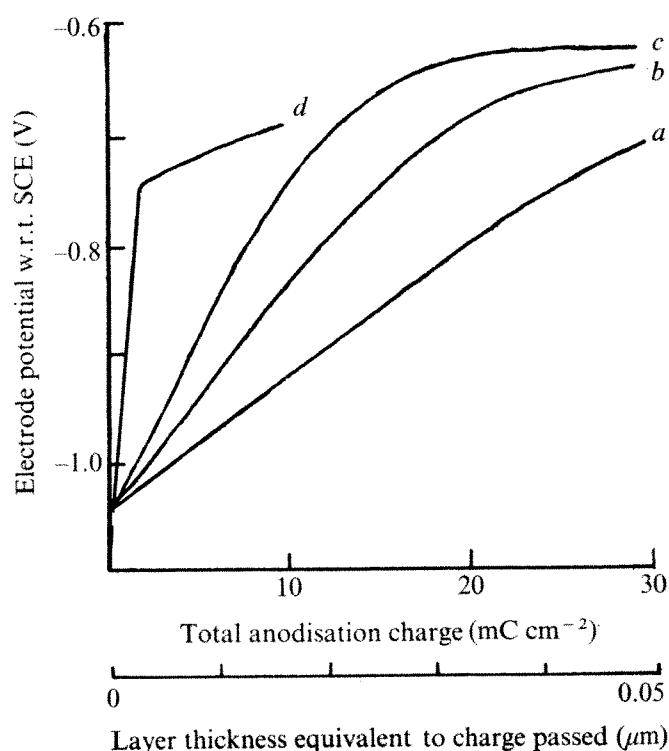
technology, which supports the present commercial solar cells, to alternative systems incorporating less pure, polycrystalline materials, deposited as thin layers to avoid wastage of expensive elements. This letter describes the operation of a novel system which may be constructed from relatively inexpensive materials, yet which converts solar radiation into electrical energy with an efficiency greater than some similar systems previously reported.

Considering the replacement of single crystals by polycrystalline materials, we find that grain boundaries can contribute to the following major limitations to the ultimate cell efficiency: first, recombination of photogenerated electron-hole pairs occurs if the optical absorption distance exceeds the minority carrier diffusion length, which is limited by the grain size; secondly, the formation of a well-defined p-n junction is complicated because of preferential adsorption of dopants at grain boundaries. The former may be avoided by the use of direct band-gap semiconductors, and the latter by replacement of the p-n junction with a Schottky barrier.

A recent development in Schottky barrier cells was the semiconductor-electrolyte liquid junction in which the usual metallic contact was replaced by a suitable electrolyte solution¹. Ellis *et al.*² showed the systems CdS (single crystal)-Na₂S_n solution and CdSe (single crystal)-Na₂S_n solution to be stable under illumination, in contrast to previous systems which had degraded by photochemical reactions. A similar electrolyte was seen to preserve semiconductor stability when Miller *et al.*³ constructed solar cells using anodically prepared films of CdS and Bi₂S₃. The efficiency of the single crystal CdS cell was limited by the high band-gap of CdS whereas CdSe gave a remarkable efficiency when used in etched single crystal form. Anodic Bi₂S₃, however, was found to be less efficient than anodic CdS despite its favourable band-gap. Since CdSe has been shown to be more efficient than CdS in single crystal form, it was thought that a comparison of the respective polycrystalline films would be informative.

Anodic films of CdS were prepared in constant current conditions. The Cd anode was kept in the dark and the elec-

Fig. 1 Galvanostatic anodisation of Cd in sulphide and selenide solutions. a, 1 M Na₂S, 2 μ A cm⁻²; b, 1 M Na₂S, 5 μ A cm⁻²; c, 1 M Na₂S, 10 μ A cm⁻²; d, 1 M NaOH + Se, 2 μ A cm⁻².



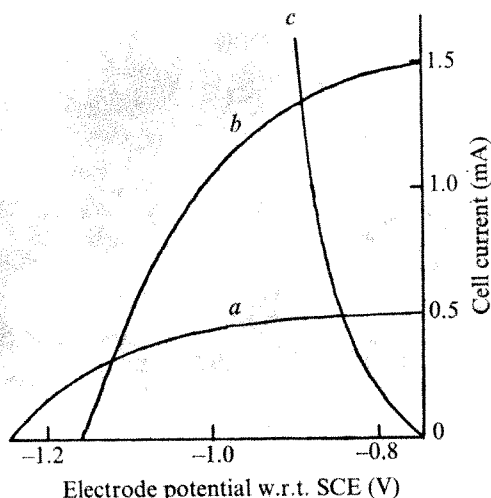


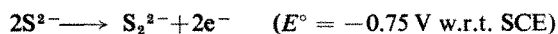
Fig. 2 Current potential characteristics: a, Illuminated CdS layer (0.5 cm^2) in molar Na_2S , 0.05 mol dm^{-3} S solution, b, Illuminated CdSe layer (0.5 cm^2) in $1 \text{ M Na}_2\text{S}$, 0.05 mol dm^{-3} S solution. c, 10 cm^2 Pt counterelectrode in molar Na_2S , 0.05 mol dm^{-3} S solution.

trolyte, molar Na_2S , was continuously purged with argon. Fig. 1 shows the anode potential with respect to a saturated calomel electrode (SCE) as a function of charge passed. The initial potential shows that the reaction was self-driving according to the equations

(for example) $\text{Cd} + \text{S}^{2-} \rightarrow \text{CdS} + 2\text{e}^-$ $E \approx -1.06 \text{ V}$ at anode

and $\text{e}^- + \text{H}_2\text{S} \rightarrow \text{HS}^- + \frac{1}{2}\text{H}_2$ $E \approx -0.75 \text{ V}$ at platinum cathode

As the CdS film grew, the anode potential rose corresponding to the field required to assist diffusion of Cd^{2+} or S^{2-} ions through the CdS lattice. The potential did not rise above -0.6 V , suggesting that some current was thereafter being diverted to a competitive reaction, for example



The thickness of the film can be estimated by applying Faraday's law to the integrated current. We conclude that growth becomes very slow after about $0.1 \mu\text{m}$. Anodisation of Cd in molar Na_2S , S at 0.5 mol dm^{-3} solution gave similar results. Fig. 1 also shows the result of anodising Cd in molar NaOH saturated with selenium—a solution known to contain polyselenide and selenide ions. This time we see that the competitive reaction occurred much earlier, so that thicknesses of above $0.01 \mu\text{m}$ were difficult to achieve by this method. Since a thicker layer would be required to efficiently absorb light, alternative methods of preparation were investigated. One method was the immersion of Cd in a boiling solution of KOH at 5 mol dm^{-3} saturated with selenium. After some hours treatment, a black layer was revealed on the Cd surface after removal of selenium in boiling Na_2S solution. We presumed this layer was CdSe.

Figure 2 shows cell current plotted against electrode potential for the cadmium sulphide and selenide films illuminated by direct sunlight in a solution of molar Na_2S , 0.05 mol dm^{-3} S. The potential was measured against an SCE immersed in the solution while the current passed through a platinum counterelectrode. In this way the potential of the photovoltaic electrode was observed undistorted by any overvoltage developed on the platinum. Approximate conversion efficiencies, assuming no overvoltage on the counterelectrode, were 0.4% for CdS and 1.0% for CdSe.

We have shown that films of CdSe may be prepared by an economically attractive process, involving no high temperatures and using a minimum amount of the less abundant element, selenium. The films were shown to have a greater efficiency than similar sulphide films as expected from band-gap considerations. Further work involving alternative preparations

of the films is showing efficiencies significantly greater than that reported here, and stability tests have as yet shown no signs of film degradation.

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Iceberg deterioration

A VERY large tabular iceberg was observed as it drifted north-east of the Grand Banks of Newfoundland during May and June 1976. When the iceberg was first sighted on 12 May 1976 it showed only minor signs of deterioration. From 12 May until last sighted on 6 June the iceberg underwent a rapid reduction of the above water surface area with the erosion largely confined to the turbulent layer associated with gravity waves. The erosion progressed along lines parallel to the structure of the iceberg as indicated by the pronounced ridge pair seen in the photographs (Fig. 1), a trellis drainage pattern, and an alternation of light and dark bands over the entire surface of the iceberg.

Photographs were taken of a large number of icebergs during the 1976 flights of the International Ice Patrol, operated by the US Coast Guard. These photographs are to be used for a study of iceberg populations off the Grand Banks of Newfoundland (Fig. 2). Black and white photographs (9-inch format) were taken from altitudes of between 300 and 3500 m.

Among the photographs obtained are a unique series of five taken of the same iceberg over a period of 25 d (Fig. 1). It is highly unusual for an iceberg to be relocated over such an extended period when positive identification is possible. Icebergs normally change their appearance so radically by a combination of calving, melting and rolling that it is impossible to positively identify them after only a few days. In this case the unusually low profile, only 4-5 m of elevation, and tabular shape maintained the iceberg in an extremely stable condition.

From 12 May to 6 June 1976 the iceberg decreased in surface area from approximately $190,000 \text{ m}^2$ to an area of $109,000 \text{ m}^2$. The rate of decrease in surface area was nearly linear (Fig. 3) and resulted from wave erosion, undercutting and minor calving. The surface water temperature was $2-4^\circ\text{C}$. Sub surface temperatures in this area typically decrease to a minimum of less than -1°C at 75-100 m depth.

Wave erosion was concentrated at those points which appear as slight irregularities in the 12 May 1976 photograph. The progressive enlargement of these embayments was the result of the local concentration of wave energy and continued bathing of the ice by the turbulent water. The embayments seemed to extend only several metres below the water's surface and have an orientation parallel to the structural features of the iceberg. The underwater shape of the iceberg is not known but it is suspected to have had a flat bottom as inferred from the long term maintenance of its top parallel to the sea surface.

The iceberg seemed to be of land ice origin. Analysis of a piece of the iceberg, recovered by the USCGC Evergreen, showed it to be fresh water ice. The characteristic air bubble bands of glacier ice were visible and surface melt tests produced a pattern of hexagonal depressions

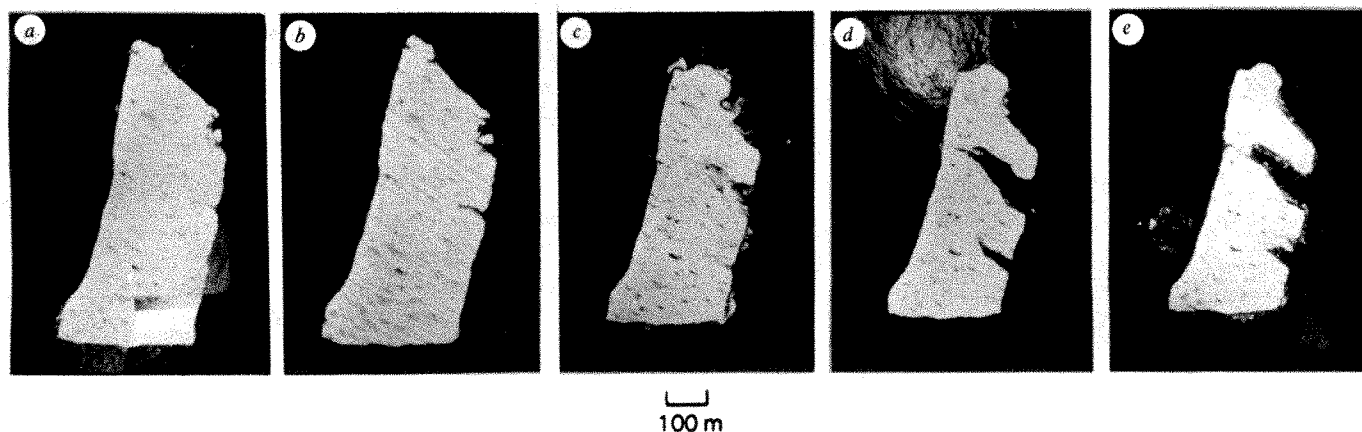


Fig. 1 The deterioration of an iceberg showing the progressive enlargement of embayments. *a*, Photograph 1708Z, 12 May 1976. 49°19.9'N, 49°33.3'W. Area 190,485 m². *b*, 1804Z, 13 May 1976. 49°28.9'N, 49°26.6'W. Area 189,126 m². *c*, 1404Z, 31 May 1976. 48°09'N, 49°34'W. Area 137,361 m². *d*, 1343Z, 4 June 1976. 47°39.7'N, 49°15.8'W. Area 121,113 m². *e*, 1812Z, 6 June 1976. 47°31'N, 49°07'W. Area 109,067 m². Scale bar, 100 m.

approximately 2 cm across which typify the crystalline melt surface texture of glacier ice. The two linear parallel ridges in the upper third of each picture of Fig. 1 are the most obvious manifestations of the ice structure. In the original photographic prints a pattern of trellis drainage can be seen which indicates structural control. There is also an alternation of light and dark bands which while quite subtle are obvious on close examination of an original photographic print. The banding is not a result of the drainage pattern since it is crossed by drainage channels in numerous locations. The band pairs do not seem to be regularly spaced nor are they perfectly linear or parallel for the width of the iceberg. We feel that such a structure is likely to be the result of flow and that the light and dark bands represent streamlines. The two parallel ridges are possibly the result of shear or flow over an irregularity of the glacial bed.

The iceberg's origin remains unknown. The Ward Hunt ice shelf on northern Ellesmere Island, Petermann Glacier in Hall Basin and Humboldt Glacier in Kane Basin are all capable of producing a thin tabular iceberg. Usually such icebergs fragment and deteriorate before they reach 50°N latitude. The last sighting of a similarly-shaped iceberg on

the Grand Banks by the International Ice Patrol was in 1964 (ref. 1). Twenty large tabular icebergs, some as long as 600 m, were sighted by the Ice Patrol aircraft in late February of 1964 between Hamilton Inlet and Cape Chidley. They appeared as far south as 44°N by early May 1964. These icebergs were thought to have their origin in ice island WH-5 which was observed to block the Kennedy Channel from the Canadian shore to Hans Island in 1963 (ref. 2).

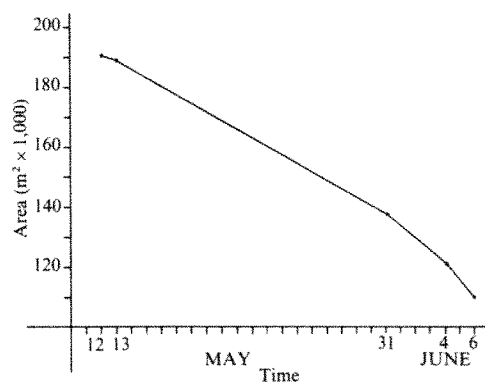


Fig. 2 Drift track of a large tabular iceberg near the Grand Banks of Newfoundland.

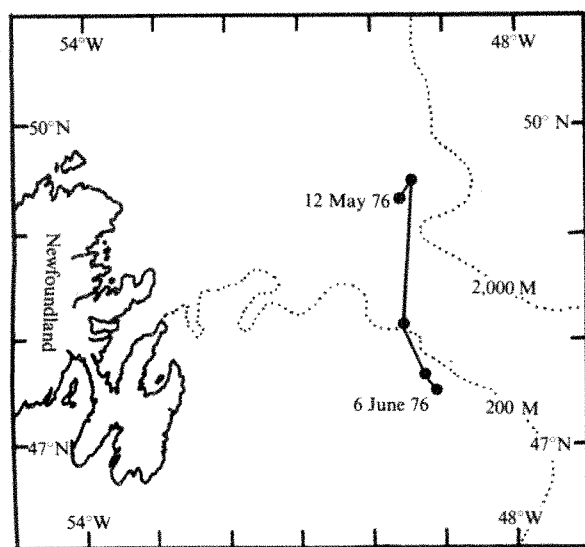


Fig. 3 The reduction of the sea level horizontal cross-sectional area as a function of time.

A low tabular berg of this size is quite unusual and fortunately rare. It represents a greater than usual hazard for surface vessels due to its lower probability of detection particularly in a seaway. The iceberg's uniqueness contributed to the attention given it by the International Ice Patrol.

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Upwelling by icebergs

A CASE is made for considering the influence of melting icebergs on the oceanographic characteristics of the Weddell Sea. In particular, the equivalent upwelling produced by melting must play a large factor in the supply of nutrients to the surface layers of this ocean. It might be, for example, that the slowly moving icebergs leave behind a trail of nutrient enhanced surface fluid. The need for testing such a hypothesis is clear.

In Antarctica the net annual yield of icebergs from the ice shelves and direct glacier streams is of order 10^{15} kg (ref. 1). The major shelf regions are the Ross, Amery and Ronne-Filchner. The latter supplies icebergs to the Weddell Sea at an annual rate of about 0.12×10^{15} kg; a similar amount is estimated to enter this sea from direct glacier streams and smaller shelves. The average density of the ice is 0.85 g cm^{-3} (ref. 2) and its temperature near the edge of the shelf of origin is of order -10°C (ref. 3).

Gordienko⁴ produced a sampling of the size distributions of icebergs off the coast of East Antarctica near the Amery ice shelf. His data show that one-half of the individual icebergs have standing heights above water of from 28 to 65 m, with frequency uniformly distributed between these limits. Average length of iceberg is about 1 km (ref. 4) although individual bergs have been reported with lengths of 45 and 70 km (ref. 5). Total iceberg accumulation in the Antarctic Oceans has been estimated at 6×10^{15} kg, which implies an average iceberg lifetime of about 6 years (ref. 1).

Studies have been reported on the economic usefulness of transporting Antarctic icebergs to lower latitudes as sources of potable water (refs 1 and 6), but no study has been reported on the role that the icebergs have in modifying the structure of the oceans in which they melt. Surface circulation in the Weddell Sea is part of a large clockwise gyre; hence the region tends to retain icebergs and the potential for modification of its near-surface structure of temperature and salinity by the icebergs is greater than that for other Antarctic locations. The results given here show that the icebergs have a significant role in causing the upwelling of waters from below the pycnocline into the shallow upper layer, and that a significant amount of buoyant potential energy is released into the upper layers of the ocean by the melt process.

The vertical structure of the upper waters of the Weddell Sea are typically as shown in Fig. 1 where the temperature and σ_t data for three stations from the 1973 Glacier cruise⁷ are given. The base of the pycnocline is at about 50 m depth. Below the pycnocline is Atlantic Warm Deep Water. An iceberg of 45 m height above water (the modal value of the Gordienko distribution) would extend some 200 m below the pycnocline and its melt water and heat sink properties would be available for modification of this water. A two-layer approximation to the representative structure shown in Fig. 1, averaged over all of the Glacier data for the Weddell Sea in Jan-Feb 1973 would be as shown in Table 1.

The potential energy released by melting below the pycnocline, and thus available for mixing between the two layers can be estimated. We assume that the dominant melting rate occurs along the sides of the iceberg. The potential energy released below the pycnocline is

$$\text{P.E.} = 0.85 \int_{25,000}^{5,000} \Delta \rho g z dz = 6.4 \times 10^9 \text{ erg per cm}^2 \text{ ice column}$$

where $\Delta \rho$ is the density difference between melt and ambient water and is of the order $25 \times 10^{-3} \text{ g cm}^{-3}$.

If melting along the bottom of the iceberg is the dominant rate, the potential energy released below the pycnocline is slightly greater than calculated above since the iceberg will sink

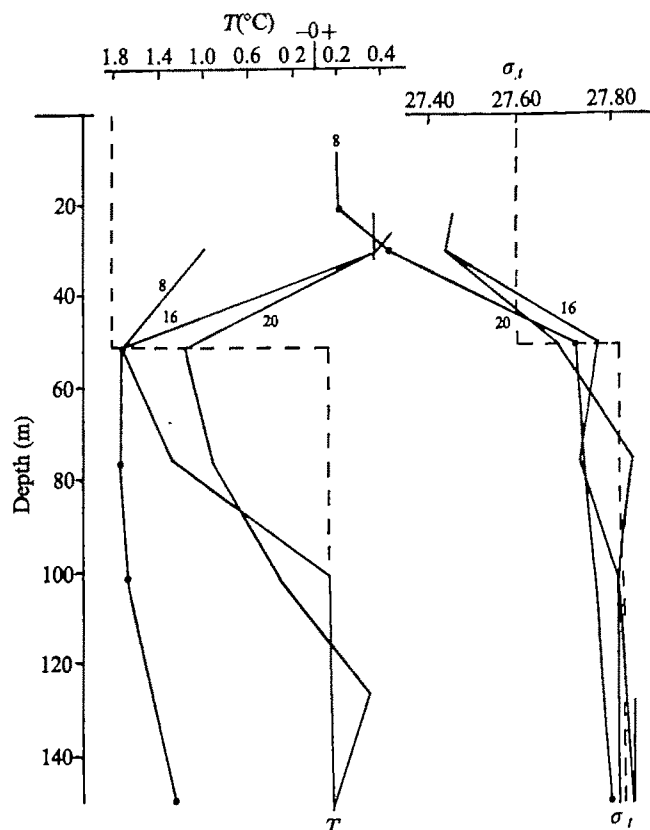


Fig. 1 Representative temperature and density structure of upper waters of the Weddell Sea⁷. Stations are located as follows: station 8: $63^\circ 26'S$, $26^\circ 45'W$; station 16: $68^\circ 50'S$, $18^\circ 20'W$; station 20: $70^\circ 10'S$, $15^\circ 10'W$. Dashed lines show a two-layer approximation based on an average of all 1973 Glacier stations in the Weddell Sea.

as the bottom melts and hence more ice melts below the pycnocline than in the case of melting along sides only. The opposite holds if melting above the water line is the dominant rate.

It is worth while to compare this energy release within the lower layer with that transferred from wind stress at the surface. The 1.2×10^{14} kg of icebergs produced annually by the Ronne-Filchner shelf alone represent $4.8 \times 10^{13} \text{ cm}^3$ of mode thickness bergs. Total energy release is then $3.1 \times 10^{23} \text{ erg yr}^{-1}$ or about $10^{15} \text{ erg s}^{-1}$. A usual wind stress figure is 1 dyn cm^{-2} . Niiler⁸ has shown that the wind related energy which passes through the mixed layer is then about $1 \text{ erg cm}^{-2} \text{ s}^{-1}$. Thus, the energy input by iceberg melt is roughly equivalent to the average wind stress input over an area of 10^6 km^2 .

Consider an iceberg of density 0.85 g cm^{-3} , area of 1 km^2 and extending 250 m into the water. That portion of the iceberg extending below the pycnocline contains $1.7 \times 10^8 \text{ m}^3$ of melt water. What is the ultimate disposition of this melt water?

We assume first that all ice melt mixes with surrounding lower layer fluid to produce a fluid of density equal to that of surface water, that is, water at 0°C , 34.00‰ and that all mixture water ascends to the surface. Let V_1 be the volume of 34.57‰ water

Table 1 Two-layer approximation to a representative temperature and density structure of the Weddell Sea averaged over all of the Glacier data for Jan.-Feb. 1973

Upper layer Thickness 50 m		Below pycnocline	
At 20 m		At 150 m	
Temperature	-1.8 to 0.6°C	Temperature	0.2°C
Salinity	33.94% { $+0.29\%$ -0.55%	Salinity	34.57%

required by the mixture to attain density equal to that of surface water, and V_2 the resultant volume of the mixture. Then

$$V_2 - V_1 = 1.7 \times 10^8$$

and

$$34.57V_1 = 34.00V_2$$

Solving for V_2 , one obtains 10^{10} m^3 of mixture water of density equal to that of surface water, for each square kilometre of iceberg. The vertical migration time of the mixture will be short relative to the melt time of the iceberg. Hence, if the iceberg melts uniformly within one year, an average vertical volume transport of mixture water into the upper layer would be of the order $3.3 \times 10^8 \text{ cm}^3 \text{ s}^{-1}$.

Vertical velocities found in the Oregon Coastal Upwelling zone are of the order $10^{-2} \text{ cm s}^{-1}$ (ref. 9). Thus the area of coastal upwelling required to produce an annual vertical transport equivalent to that of the 1 km^2 iceberg is 3.3×10^{10} or 3.3 km^2 .

Since the iceberg presumably melts at a uniform rate throughout the year, whereas Oregon upwelling is effective only 3 months each year, the coastal upwelling equivalent area may again be increased by a factor of four, to a value of 13.2 km^2 per each square kilometre of iceberg.

In contrast to coastal upwelling, iceberg upwelling is permanent. In Oregon waters, for example, some fraction of the nutrients which are contained in the upwelled water are returned to the deeper zones unused at the time of subsequent sinking of the upwelled waters at the frontal zone. We estimate a 50% utilisation of nutrients in the Oregon case (Lawrence F. Small, personal communication). Then one must again increase the coastal upwelling equivalent area by a factor of two, to 26.3 km^2 .

For an annual iceberg production in the Weddell Sea region of $1.2 \times 10^{14} \text{ kg}$, or $1.2 \times 10^{11} \text{ m}^3$, the number of square kilometres of mode sized icebergs produced and melted each year is 1,400. In terms of area of upwelling equivalent to that of Oregon coastal upwelling, the Weddell Sea has $1,400 \times 26.3$ or $36,800 \text{ km}^2$ of such area. Stated another way, since the Oregon coastal upwelling region extends some 10 km to sea, the upwelling produced in the Weddell Sea by melting icebergs is equivalent to some 3,700 km of Oregon coastline, a distance approximately equal to the length of coastline of the North American continent from Acapulco, Mexico to Ketchikan, Alaska.

The importance, as shown above, of upwelling by icebergs in Antarctica is striking, and one immediately returns to question the validity of the underlying assumptions. Two of the assumptions are critical: first, that melt water entrains sufficient ambient fluid to acquire a density equal to that of fluid in the surface layer, and second, that all of the mixture reaches the surface layer. A key element in evaluating the validity is to examine the Grashoff number which characterises a free convection regime along a vertical wall at which buoyant energy is generated.

For a vertical, heated wall in air, the Grashoff number is given by¹⁰

$$N_{Gr} = \frac{l^3 \rho^2}{\mu^2} g \beta (T_f - T)$$

where l is the characteristic vertical length (the depth of the iceberg below the pycnocline), ρ is the density, g is gravity, β is the thermal expansion coefficient, T_f is the fluid temperature in the field far from the wall, T is the wall temperature, and μ is the fluid viscosity. In the case of iceberg melt, the Grashoff number is predominantly affected by the density difference between that of melt water, ρ , and that of surrounding saline sea water, ρ_f : hence

$$N_{Gr} = \frac{l^3 \rho^2 g}{\mu^2} \left(\frac{1}{\rho} (\rho_f - \rho) \right)$$

For a density difference ($\rho_f - \rho$) of 0.025 g cm^{-3} , one obtains a Grashoff number of the order of 10^{17} . This is an extremely high value which places the vertical flow along the sides of the melting iceberg clearly within a fully turbulent state. For this reason, it is reasonable to assume the conditions stated above.

An iceberg with a plane bottom coincident with a level surface will not cause free vertical convection since the melt fluid along the bottom is constrained from rising by the iceberg itself. If the bottom is tilted relative to a level surface, melt fluid will experience vertical acceleration although the Grashoff number would be smaller than that which characterises flow along vertical sides. Clearly, some bottom melting must occur, hence the released energy estimation previously stated is conservative.

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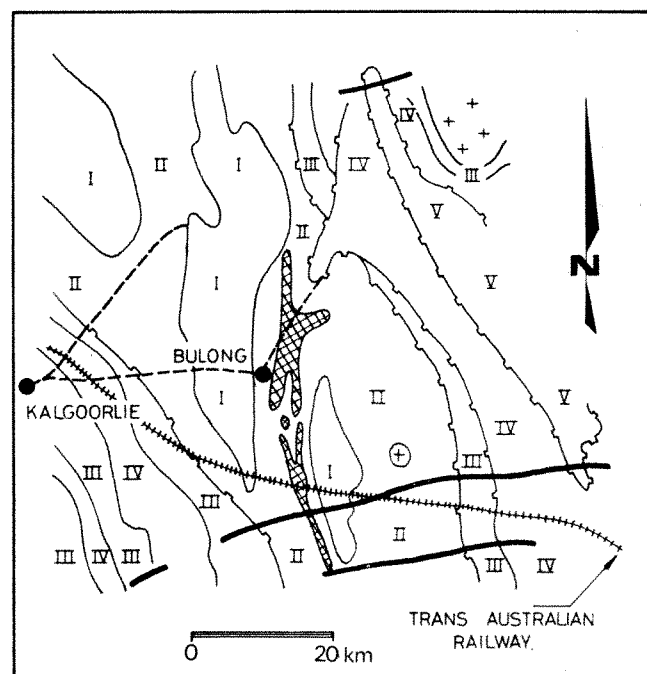
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New type of igneous layering due to fractionation of Archaean ultramafic magma

RESEARCH into the petrogenesis of the Bulong Complex¹, near Kalgoorlie, Western Australia (Fig. 1), has indicated that it is composed largely of layered ultramafic cumulates which exhibit both phase and cryptic layering indicative of low pressure fractionation of Archaean ultramafic magma.

The Bulong Complex covers some 200 km^2 but its greatest stratigraphic thickness is only 1.1 km. Detailed research¹ has indicated that the complex originated as a series of liquid-rich, ultramafic magma pulses which were introduced, largely concordantly, into an extensive, but narrow, stratigraphic zone of the originally flat-lying, host formation.

Fig. 1 Geological setting of the Bulong Complex (hatched areas).



Smaller ultramafic pulses either froze to form homogeneous olivine-rich, 'quench-texture' sills or underwent flowage differentiation, before freezing, to form crudely zoned ultramafic sills with olivine-rich cores. Larger ultramafic pulses, on the other hand, differentiated *in situ*, largely by gravitational processes, to produce layered olivine-rich sills which are capped in part by layered pyroxene and pyroxene-plagioclase bearing horizons. During fractionation, small pulses of derivative ultramafic to acid magma were probably tapped off at various stages and intruded about the top of the complex to form the numerous small sills and dykes which are now observed in that position. When igneous activity had virtually ceased the complex and its host rocks were regionally folded (three periods of variably tight to open folding are evident and the complex is even locally overturned) and metamorphosed to middle greenschist grade^{1,3}.

Layered, olivine-rich sills constitute most of the complex and in most localities 3 to 5 sills, stacked one on top of the other and separated in part by thin sheets of country rock, are evident. The sills mainly range in thickness from 350 to 400 m and although all have been deformed, serpentinised and regionally metamorphosed, primary textural features are well preserved and primary mineral relics are not uncommon. The least altered sills contain thin (1–3 m thick) remnant basal 'chilled border' zones composed of olivine-rich, porphyritic spinifex rock⁴ (the presumed quenched starting liquid)¹. About 95%, or more, of the overlying layered rocks are olivine cumulates, which contain accessory amounts of cumulus chromite, in which the proportion of orthocumulus material increases gradually from about 5%, or less, in lowermost parts to 30%, or more, in uppermost parts. The orthocumulus minerals in decreasing order of abundance, are clinopyroxene, orthopyroxene and plagioclase, although hornblende and biotite also occur as orthocumulus phases near the tops of some sills. The thick, layered olivine-(chromite) cumulate zones are capped in part by thin orthopyroxene cumulate layers which are in turn capped successively by thin orthopyroxene-clinopyroxene and plagioclase-clinopyroxene-orthopyroxene cumulate layers. In at least one of the sills a thin chromitite (chromite cumulate) layer is located directly beneath the orthopyroxene cumulate layer. All sills exhibit normal cryptic layering delineated by slight, but consistent iron enrichment in relict olivine (Fo 93 to Fo 86), chromite ($\Sigma\text{FeO}=18$ to 36 wt%; $a_0=8.26$ to 8.31 Å) and clinopyroxene ($\text{Ca}_{15}\text{Mg}_{51}\text{Fe}_4$ to $\text{Ca}_{12}\text{Mg}_{49}\text{Fe}_9$) with increasing stratigraphic height. Clinopyroxene occurs mainly as an intercumulus phase but topmost clinopyroxenes are cumulus and co-exist both with cumulus orthopyroxene (En15) and cumulus plagioclase (An 80 to An 85). Within the main layered olivine-(chromite) cumulate zones there is a marked upward increase in whole rock Ti (1,000–3,000 p.p.m.) and P (50–200+ p.p.m.) content and a marked decrease in Ni (3,500–1,000 p.p.m.) content. This is largely a reflection of the gradual upward increase in the proportion of orthocumulus material¹.

The field evidence^{1,2} suggests that at the time of emplacement of the layered sills the confining pressure due to overlying rocks was only about 1 to 2 kbar and this is supported by (1) high CaO content (0.3–0.5 wt%) in relict olivine^{5,6}, (2) moderate to high $\text{Al}^{\text{IV}}/\text{Al}^{\text{VI}}$ (>2) ratios⁷ and TiO_2 contents (0.2–0.6%) (ref. 8) in relict clinopyroxene, (3) abundant evidence for reaction between settled olivine and trapped liquid⁹ before onset of intercumulus pyroxene crystallisation and (4) by a general lack of evidence for subsolidus reaction between cumulus olivine and intercumulus plagioclase¹⁰. Furthermore, simplified low-pressure (1–2 kbar) phase relations for the system olivine-clinopyroxene-plagioclase-quartz¹¹ accurately predict the observed crystallisation sequence obtained using the mean composition of the basal, chilled contact rocks¹. The one

known chromite horizon is thought to have simply formed as a 'lag deposit', partly because of the peritectic relation between orthopyroxene and chromite¹² (that is, orthopyroxene precipitation suppresses chromite precipitation) and because of the relative settling rates of olivine (coarse-grained) and chromite (very fine-grained)¹⁴.

Liquidus phase relations for natural ultramafic rock of similar composition to that of the chilled contact rock (Table 1)¹³ clearly indicate that the ultramafic magma pulses which gave rise to the Bulong Complex were low in water content (1–2 wt%) and were also emplaced at temperatures slightly in excess of 1,650 °C. Finally, the TiO_2 content of the mean chilled contact rock (Table 1) suggests that it was derived by approximately 60% partial melting of an Archaean upper mantle source¹⁵.

Table 1 Chemical analyses of ultramafic quench rocks (recalculated anhydrous)

	Ultramafic quench rock from South African Archaean flow ¹³	Mean basal chilled contact rock in Bulong Complex (5 analyses)
	a	b
SiO_2	46.5	43.4
TiO_2	0.2	~0.25
Al_2O_3	3.6	4.5
ΣFeO	10.3	7.0
MgO	33.0	39.0
CaO	5.0	3.1
Na_2O	0.5	0.4
K_2O	0.2	0.2
Cr_2O_3	0.4	0.4
NiO	0.3	0.3
$\text{CaO}/\text{Al}_2\text{O}_3$	1.4	0.7

Discovery of the phase and cryptic layering reported here has not only extended our knowledge of the spectrum of Archaean ultramafic rock types, which up until now has included various types of zoned and unzoned non-layered sills and flows (refs 4, 15–17), but has also broadened our knowledge of the total spectrum of layered igneous rocks¹⁸. A detailed account of phase and cryptic layering in the Bulong Complex is in preparation.

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Effects on biological evolution of changes in ocean chemistry

PERHAPS the most perplexing problem of biological evolution involves explanation of why diversification of life began only after 85% of the lifetime of the Earth while simple life seems to have been present for virtually all of geological time. Recent observations¹⁻² on the presence of complex organic species in meteoritic debris and in the interstellar medium show that some of the basic biological building blocks may have been available from the earliest times. Chamberlain and Marland³ have commented on the biological consequences of the Hargraves⁴ model of ocean-crust evolution while others⁵⁻⁶ have reassessed evidence on atmospheric evolution and indicated that changes in oxygen partial pressure may not have been critical to the diversification of life. In this note I suggest that important changes in ocean chemistry must have occurred if our current models of crustal evolution and igneous events have any validity. The evolution of complex life forms requires a high degree of environmental stability and constancy of chemical environment. It is suggested that this condition is closely linked to the spacing of major volcanic centres, and that the modern long wavelength pattern of ocean ridges and subduction zones was necessary to achieve this stability. The distribution of ophiolites or ocean-floor structures of modern type⁷ indicates that the present pattern may have been initiated about one billion years ago. Furthermore, large scale continental emergence⁴ may have been necessary to provide oceans with stable chemistry.

There is much evidence to support the Hargraves⁴ model of early globe-encircling oceans and slow continental emergence. The ocean mass may have been greater⁸. Further evidence shows that with an early hotter earth, volcanic activity was more intense and that centres of volcanic activity were closely spaced with a wavelength pattern of hundreds not thousands of kilometres. We also know that the cooling of submarine igneous bodies involves convective flow of seawater and that the chemistry of the cold inflow and hotter discharge waters is quite different. Thus, early oceans would tend to reflect volcanogenic discharge more than continental runoff. As such, chemistry would be erratic and frequently concentrations of typical easily transported metals like Cu, Hg, Pb, Zn, Mn, Fe, As, could be large as in the modern Red Sea deeps. (The Red Sea illustrates⁹ the toxic effects of such discharge.)

For the present earth, continental runoff amounts to about 3×10^{10} g yr⁻¹ while the ocean mass is 1.4×10^{24} g. Submarine volcanic activity associated with the ocean-floor spreading process currently adds some 3×10^{16} g of basalt liquids to the ocean annually. This liquid at 1,200 °C cools by convective flow of ocean water. Sites of discharge will cause local changes in chemistry as seen in the Red Sea and many other places^{10,11}. The energy input at modern ocean ridges (4×10^{18} J yr⁻¹) could heat 10^{17} g of sea water to 100 °C. Thus, at present, hydrothermally modified ocean water might approach 1% of the volume of continental runoff. If this water contains any chemical species at much higher concentrations than in river water, it may influence the balance of ocean waters.

Elements that are present at much higher levels than in normal ground waters (100 times) include a host of those which can be toxic to complex life forms (As, Hg, Cu, Zn, Mn, Fe, Pb). Furthermore, if there was little land above sea level in the Archaean, the input of metal absorbing clay mineral would be reduced, and this detoxification process would be less effective¹².

At present, while basaltic mantle-derived volcanic processes are concentrated in the submarine environment, subduction andesite-granite igneous activity is concentrated

on the continents and if convective cooling occurs, it involves less saline waters which in general transport less metal. But in the Archaean, nearly all igneous activity was submarine. The huge granite bodies of the Archaean could generate sustained submarine discharge on a scale not seen today. Imagine the consequences of emplacing the Sierra Nevada Batholith in a submarine environment.

Thus, the Archaean ocean floor may have been populated by closely spaced volcanic centres with an igneous intensity an order of magnitude more than known today¹³. From such centres there would have been an intense discharge of toxic elements and hydrogen into deep basins. In such an environment, one would have expected only the most simple and versatile life forms to survive.

Runcorn¹⁴ has discussed the possibility of quantised changes in mantle convection associated with cooling. If cells have become larger with time, then the current convection pattern may have been necessary for the recent biological diversifications. Fluctuating environmental pollution caused by closely spaced centres of intense submarine volcanism may have inhibited complex biological evolution for most of earth history.

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Interaction between effects of vesicular-arbuscular mycorrhiza and fertiliser phosphorus on yields of potatoes in the field

MANY experiments in controlled environments have shown that vesicular-arbuscular (VA) mycorrhizas may benefit the phosphorus uptake and growth of plants¹⁻⁴, depending on the nature and level of indigenous inoculum, the phosphorus status of the soil and other factors⁵⁻⁷. But there have been few reports of attempts to obtain larger crops, or reduce phosphorus fertiliser requirement, by manipulation of VA mycorrhizal infection in field crops⁸⁻¹². We report here the effect on potatoes of an artificial increase in the level of VA infection. Potatoes are known to have VA mycorrhizas¹³⁻¹⁶, and their sparse rooting systems¹⁷ and heavy phosphorus demand suggest that they should respond to infection. This is the first report of a successful field inoculation experiment on a major field crop in temperate zone conditions.

The method of inoculation depended on observations, made in a separate experiment, on the changes of spore number and extent of infection caused by different cropping sequences. Cropping with cereals tended to induce high spore density, with heavy infection of subsequent crops, whereas if the area was left fallow there was a rapid decline in spore density and subsequent level of infection (Table 1).

The potato experiment was sited on an area left fallow for 2 yr, so that the spore density was only about one per ml. The inoculum consisted of topsoil from nearby plots,

Table 1 Effect of crop sequence on VA mycorrhizas in barley, together with spore density in soil

Growing season		Infection in August (% of root length)	Spore density (no. per ml of soil, fraction retained on 45-µm sieve)	
			Spring	Autumn
1975	Continuous barley (4 yr)	39	29	21
	Barley after 1 yr fallow	21	18	10
1976	Continuous barley (5 yr)	19	17	17
	Barley after 2 yr fallow	5	4	Not measured

on the same soil series, on which barley had been grown for 3 yr, and which contained 13 spores per ml. The most frequent spore type was identified as *Glomus macrocarpus*¹⁸. The experimental plots were 3.05 m × 3 m, and received ammonium nitrate (437 kg ha⁻¹) and potassium chloride (502 kg ha⁻¹) fertiliser broadcast before planting of Pentland Javelin seed potatoes. They were treated with triple superphosphate (0 or 481 kg ha⁻¹), combined factorially with presence or absence of soil inoculum. The latter was applied down the furrows at about 3 kg m⁻². It contained available (Olsen) phosphorus at 15 µg g⁻¹, compared with 7 µg g⁻¹ in the plot soil; the amount of added available phosphorus was therefore extremely small compared with any useful fertiliser dressing. Uninoculated plots received the same amount of topsoil from the fallowed area. There were four replicates. During the period of growth, soil core samples were taken, the roots in them were washed out and percentage infection was determined¹⁹.

Infection spread most between the first two root samplings (Table 2). Infection with soil inoculum was much greater than the very low control level but, as expected, phosphorus fertiliser suppressed it considerably. No treatment had a significant effect on phosphorus in leaflets sampled on 12 July (Table 2), possibly because leaf senescence was becoming general.

At harvest, fertiliser phosphorus increased yield, but least so in the presence of cereal soil inoculum. Interaction between inoculum and fertiliser phosphorus is indicated by the large increase in yield caused by inoculum, but only in the absence of phosphorus fertiliser.

An important purpose of our trial was to test the effectiveness of a relatively small amount of soil as inoculum for VA mycorrhizal infection. Rich and Bird¹⁰ reported successful use of imported soil containing twice as many spores as the soil at the experimental site, whereas our soil inoculum had more than 10 times as many spores as the field soil, but was used in smaller quantities. The micro-

biological population of the inoculum soil and the plot soil must have differed in many respects, including their content of pathogenic organisms, because of their different cropping history. Other organisms in the inoculum soil may have infected or altered the crop, but the increase in mycorrhizal infection, and the interaction with phosphorus fertiliser, leaves little doubt that it was principally VA organisms which affected the yield.

Our experiments illustrate two important points. First, the effects of cropping rotation on the VA infection of subsequent crops must always be considered²⁰. Second, the phosphorus status, fertiliser response and economics of the use of fertiliser for mycorrhiza-forming field crops may depend greatly on the degree of infection, and work on these may be incomplete unless infection is measured. The frequent difficulty of correlating phosphorus requirement of a crop and soil analytical results may also be due to differences in VA infection.

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Table 2 Effect of inoculum and fertiliser phosphorus on mycorrhizal infection, foliar phosphorus and tuber yield

		Control seedbed (fallow soil)		Inoculum seedbed (cereal soil)	
Triple superphosphate (kg ha ⁻¹)		0	481	0	481
% Mycorrhizal infection*					
	22 June	0.5 a	0.9 ac	8.3 b	4.0 bc
	12 July	4.6 a	1.6 a	24.3 b	2.6 a
	2 August	3.2 ac	1.2 a	24.5 b	5.9 c
% Phosphorus concentration in leaves					
	12 July	0.16 a	0.19 a	0.17 a	0.17 a
Tuber yield (kg per plot)					
	3 August	6.15 a	9.49 b	7.39 c	8.80 b

Means in any one row with the same letter do not differ significantly at $P = 0.05$.

*Means compared as arcsin transforms from percentage infection.

Chemical activation of host defence mechanisms as a basis for crop protection

THE possibility that a chemical for plant disease control might function by activating the natural resistance mechanisms of the host has been suggested previously¹, but there are few well-documented examples. We report here that 2,2-dichloro-3,3-dimethyl cyclopropane carboxylic acid (WL 28325) may exert its systemic fungicidal activity against the rice blast disease (caused by the fungus *Piricularia oryzae*) in this way.

It is unlikely that the WL 28325-induced resistance of rice to blast can be attributed to either the direct action of the fungicide on the parasite or the production of fungitoxic derivatives within the plant². There is, however, evidence to suggest that WL 28325 acts by sensitising the host such that subsequent inoculation with *P. oryzae* results in a resistant reaction. Both the natural and the WL 28325-induced resistance of rice involve a hypersensitive type of browning reaction (as is commonly found in incompatible plant-fungus interactions) presumed to be a localised accumulation of melanoid pigments in the plant cells resulting from the peroxidase-mediated oxidation of phenols. This response is accentuated and accelerated by previous treatment of the plants through the roots with WL 28325 (ref. 3).

Microscopic observations of the infection process on

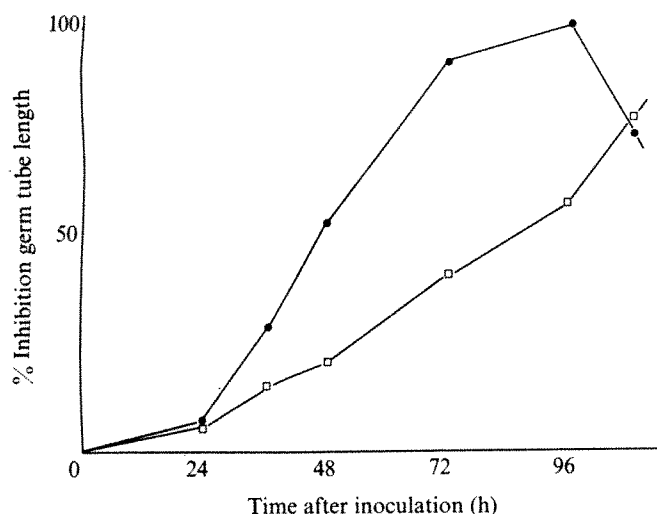


Fig. 1 Effect of WL 28325 treatment and *P. oryzae* infection on the inhibitory activity of drop diffusates against germ tube growth. ●, WL 28325-treated and infected; ○, untreated and infected. Each point is the mean of three replicate samples.

leaves showed that germination and appressorium formation by spores of *P. oryzae* were similar on WL 28325-treated and untreated plants. The internal development of the disease was examined in toluidine blue stained transverse sections of wounds on leaves of treated and untreated plants. In untreated leaves examined 48 h after inoculation, fungal hyphae were observed spreading throughout the vascular bundles of the inoculated wound. In the following 40 h the hyphae increased in number and spread into the tissue surrounding the wounded area. Hyphae were rarely observed in inoculated wounds of treated leaves, however, even 96 h after inoculation. This suggests that WL 28325-induced resistance of rice to *P. oryzae* might involve a more direct suppression of fungal growth rather than a physical restriction of the fungus to the inoculated area by the brown melanoid pigments as suggested previously³.

This possibility was substantiated when infection droplets were removed at intervals from the wounds on treated and untreated leaves and tested against germ tube growth of *P. oryzae*. When compared with spores germinated in distilled water, inhibition of germ tube growth was evident in diffusates collected from treated, infected leaves (Fig. 1). Diffusates collected from untreated, infected leaves were considerably less inhibitory while those collected from uninfected leaves, either treated or untreated, showed no antifungal activity. Repeated experiments confirmed that this antifungal activity induced by infection with *P. oryzae*, was enhanced by treatment of the plants with WL 28325 before inoculation.

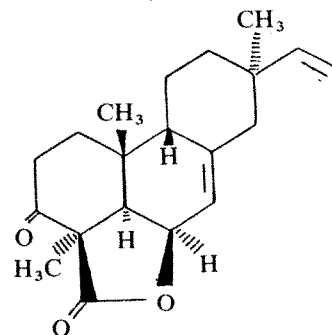
Similar results were obtained using ethanolic extracts of infected leaves rather than drop diffusates. The extracts were subjected to thin-layer chromatography (silica gel; solvent system, chloroform-ethanol (95:5v/v) followed by a direct bioassay for antifungal compounds using *Cladosporium cucumerinum*⁴. This revealed two major antifungal zones in chromatograms of extracts of infected leaves. Chromatograms of extracts of uninfected leaves showed no such inhibitory areas. Similar antifungal activity could be induced by ultraviolet irradiation of either leaves or dark-grown coleoptiles of rice. In these conditions antifungal activity was not influenced by treatment of the plants with WL 28325. Elution of the antifungal zones from the chromatograms showed that the areas of the chromatograms which inhibited *C. cucumerinum* also inhibited *P. oryzae*.

Fungal infection thus induces the production of antifungal compounds which may be defined as phytoalexins—antimicrobial compounds formed by plants in response to

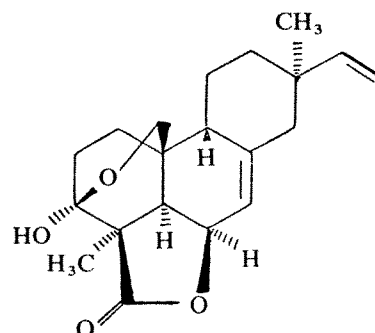
injury, physiological stimuli or the presence of infectious agents. As the phytoalexins are induced in untreated rice plants following ultraviolet irradiation they are clearly of host and not fungal origin and they are not antifungal derivatives of WL 28325.

The two major phytoalexins were isolated in small quantities as chromatographically homogeneous amorphous solids from ultraviolet-irradiated dark-grown rice coleoptiles by column chromatography on Sephadex LH-20 and preparative thin-layer chromatography on silica gel. Electron impact (EI) and field desorption (FD) mass spectrometry of the less polar compound showed a molecular ion at m/e 314. The more polar compound showed no clearly recognisable molecular ion by EI mass spectrometry but by FD mass spectrometry a presumed molecular ion at m/e 330 together with a strong $M+1$ ion was obtained. Detailed analysis of the 270-MHz proton magnetic resonance (PMR) spectra indicated their probable identity with momilactones A (I) and B (II). These two 9- β -pimaradiene diterpenes have been isolated as plant growth inhibitors from rice husks^{5,6}. Comparisons by combined gas-liquid chromatography (GLC)-mass and PMR spectroscopy of the phytoalexins isolated from rice with authentic samples subsequently confirmed their identity with the momilactones A and B. Optical rotation measurements showed that they also have identical stereochemistry. Yields of the momilactones A and B isolated from ultraviolet-irradiated coleoptiles were 3.8 and 0.9 mg per kg fresh weight respectively while estimated recoveries were 70 and 50% of the momilactone content of crude extracts. Combined GLC-mass spectroscopy confirmed the presence of the momilactones A and B in extracts of infected leaves and these two compounds account for all the antifungal activity of the crude extracts. Concentrations giving 50% inhibition of germ tube growth of *P. oryzae* for the momilactones A and B were 5 and 1 $\mu\text{g ml}^{-1}$ respectively.

We believe that this report is the first in which phytoalexins have been characterised from a member of the Gramineae, although there have been previous indications that phytoalexins may be produced by rice⁷ and



(I) Momilactone A



(II) Momilactone B

barley⁸. The diterpene nature of the momilactones is a significant departure from all previously described phytoalexins, these being mostly phenols or sesquiterpenes⁹. A diterpene hydrocarbon from *Ricinus communis*, casbene, may have some of the properties of a phytoalexin although its production in response to infection was only reported for cell-free extracts¹⁰.

While certain fungicides are themselves capable of eliciting phytoalexin production¹¹, the activity of WL 28325 is unique in that the compound itself does not stimulate phytoalexin production but rather it increases the capacity of rice to synthesise phytoalexins in response to infection—this may be the basis for its disease-protectant properties. It is hoped that future studies on the effects of WL 28325 on the host-parasite relationship in the rice blast disease will not only provide further insight into the processes of natural resistance of rice to disease but will also indicate ways in which host defence mechanisms can be manipulated to control plant disease.

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Stimulation of egg-laying by nerve extracts in slugs

LITTLE is known about the mechanism of egg-laying in mollusca, although their reproduction is under endocrine control. In the freshwater snail *Lymnaea stagnalis*, cauterisation of the neurosecretory caudodorsal cells of the cerebral ganglia causes cessation of oviposition. Subsequent injection of a homogenate of cerebral commissures induces oviposition in all snails with caudodorsal cells. This suggests that the caudodorsal cells produce a hormone, which

stimulates oviposition¹. Kupfermann² induced ovulation and oviposition in *Aplysia californica* by injection of a homogenate of neurosecretory cells, the so-called bag cells, from the abdominal ganglion of this species. Although the morphology of the reproductive system has been studied in various terrestrial slugs^{3–7}, there are conflicting reports about the control of reproduction. I report here that homogenates of the central ganglia and tentacles, which are the sites of neurosecretory cells^{8,9}, stimulate egg-laying in the slugs, *Deroceras reticulatus* (= *Agriolimax reticulatus*) and *Limax flavus*.

Egg-laying in *D. reticulatus* occurs throughout the year, reaching peaks in April and October while in *L. flavus* the breeding season is May to November. I used slugs during their breeding season. Brains and tentacles were extirpated and homogenised in physiological saline. Then they were injected into the body cavity at a dose of four or five homogenised organs per slug. The rate of fertilisation, as represented by the hatching rate, of oviposited eggs was also examined. After 7 d oviposited eggs were counted.

The results are shown in Table 1. Brain hormone (homogenate of central ganglia) stimulated egg-laying and tentacular hormone (homogenate of tentacles) inhibited egg-laying in both species. To investigate the nature of the active principles in the homogenates, the effects of boiling and dialysis of each sample were studied. Table 1 shows that the results were similar to those for crude extracts—the treated homogenates of ganglia and tentacles still stimulated or inhibited egg-laying. The rate of fertilisation in the group given tentacular hormone was lower than that in the group given brain hormone or in the control groups. Table 2 shows the results of similar experiments on slugs with tentacles extirpated. Removal of tentacles induced a large increase in the number of eggs laid and an increase in the rate of fertilisation in both species. Results of hormone treatment were similar to those observed with intact slugs of both species. Accelerated oviposition and a high rate of fertilisation induced by removal of tentacles was inhibited by injection of tentacular hormone. The injection of brain hormone into slugs without tentacles induced still greater egg-laying and fertilisation in both species. Both hormones remained active in spite of boiling or dialysis. The minimum time between injection and the onset of egg-laying was 6 h. But the effects of the hormones continued for about 7 d. Egg-laying usually commenced 12 h after treatment. In *Lymnaea stagnalis*¹, the mean response latency was 131 min, *Aplysia californica*², it was 72 min but there was a seasonal fluctuation in amount of active principle and/or the ability to respond¹⁴. In *Lymnaea stagnalis*, injection of cerebral commissures into intact snails induced oviposition in most but not all recipients. In my experiments, some individual recipient slugs did not respond to the injection of brain extract.

These results suggest that brain hormone stimulates egg-laying directly, while tentacular hormone inhibits the brain

Table 1 Effects of the injection of brain and tentacular homogenates on oviposition in the slug, *Deroceras reticulatus* (D) and *Limax flavus* (L)

Materials injected	No. of slugs used		No. of eggs oviposited (rate of fertilisation)		Average no. of eggs per slug	
	D	L	D	L	D	L
Brain homogenates						
Crude	20	20	510 (72%)	420 (76%)	25.5	21.0
Heated	14	14	329	259	23.5	18.5
Dialysed	14	14	308	266	22.0	19.0
Tentacular homogenates						
Crude	20	20	210 (69%)	110 (74%)	10.5	5.5
Heated	14	14	77	105	5.5	7.5
Dialysed	14	14	63	112	4.5	8.0
Control (Physiological saline)	14	14	224 (85%)	189 (87%)	16.0	13.5

Table 2 Effects of the injection of brain and tentacular homogenates on oviposition in the slug, *Deroceras reticulatus* (D) and *Limax flavus* (L) without tentacles

Materials injected	No. of slugs used		No. of eggs oviposited (rate of fertilisation)		Average no. of eggs per slug	
	D	L	D	L	D	L
Brain homogenates						
Crude	20	20	640 (94%)	461 (93%)	32.0	23.0
Heated	14	14	406	280	29.0	20.0
Dialysed	14	14	427	273	30.5	19.5
Tentacular homogenates						
Crude	20	20	140 (63%)	120 (71%)	7.0	6.0
Heated	14	14	119	70	8.5	5.0
Dialysed	14	14	105	98	7.5	7.0
Control						
Tentacles cut off	14	14	350 (88%)	259 (81%)	25.0	18.5
Intact	12	12	144 (82%)	108 (78%)	12.0	9.0

hormone. The role of these hormones in fertilisation remains unclear.

Tentacles have been shown to be neuroendocrine organ⁸⁻¹². In the tentacle, the tentacular ganglion is located close to the eye. It is connected with the procerebrum by the tentacular nerve. It seems likely that the tentacular hormone inhibits the activity of the neurosecretory cells of the central ganglia and as a result, egg-laying is inhibited. This resembles the hypothalamo-hypophysis-gonadal system in vertebrates. As the eye is located near the neurosecretory cells of the optic tentacle, environmental stimuli such as light and day-length, may influence the release of tentacular hormone.

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Differential pigmentation in the sexual phase of *Coelomomyces*

THE fungal mosquito parasite, *Coelomomyces dodgei* (Chytridiomycetes: Blastocladales), produces gametophytes and gametangia of two different colours, light amber and bright orange, in the intermediate copepod host, *Cyclops vernalis*. Each gametangium produces gametes of a corresponding colour. Experimental evidence is presented here which indicates that these differentially pigmented gametes are of opposite mating types, the light amber being female and the bright orange being male, and that zygotes capable of infecting mosquitoes, are produced by their fusion. This differential pigmentation provides a valuable marker for identifying gametes of opposite mating type for experimental purposes, and additionally provides further evidence for a phylogenetic relationship between *Coelomomyces* and certain other genera of the order Blastocladales.

Since the 1930s, fungi of the genus *Coelomomyces* have

been considered potential biological control agents for mosquitoes because of their well documented ability to produce epizootics in larval populations¹⁻³. This potential has not been realised or even accurately evaluated because of the difficulties of establishing laboratory cultures for detailed investigations. The primary reason for these difficulties became apparent during the past few years with the discovery that *C. psorophorae*^{4,5} and *C. punctatus*^{6,7}, parasites respectively of the mosquitoes *Culiseta inornata* and *Anopheles quadrimaculatus*, have alternate stages which develop in an intermediate host, the copepod, *Cyclops vernalis*. This finding led to the development of routine reproducible methods for the establishment of *in vivo* laboratory cultures of *C. psorophorae* and *C. punctatus* and more recently has resulted in the establishment of an *in vivo* culture of *C. dodgei*⁸ using *A. quadrimaculatus* and *Cy. vernalis* as alternating hosts.

Two of the above studies^{6,8} have also provided substantial evidence that these three species of *Coelomomyces* have an Euallomycetes type of life cycle with sporophyte and gametophyte generations alternating between the mosquito and copepod host, respectively. In essence, the evidence suggests the following general life cycle, although many details remain to be resolved, including precisely when meiosis occurs. The sporophyte in the mosquito larva produces meiosporangia which release meiospores after death of the host. The meiospores, in turn, infect copepods and develop into gametophytic thalli which subsequently become gametangia and produce gametes. Gametes fuse forming zygotes which infect mosquitoes and produce a sporophyte, thereby completing the life cycle. This cycle was first elucidated by Whisler *et al.*⁹ working with *C. psorophorae* and later by Federici and Chapman⁸ studying *C. dodgei*. The latter authors also observed gametophytes and gametes of two different colour types, light amber and bright orange, which apparently do not occur in *C. psorophorae*, and suggested that this differential pigmentation corresponded with opposite mating types. The present report provides experimental evidence for this hypothesis.

Copepods of *Cy. vernalis* were infected with *C. dodgei* using methods similar to those of Federici and Chapman⁸. Several hundred copepods were combined with about 5,000 dehiscing meiosporangia in 1 l of deionised water to which approximately 50 mg each of brewer's yeast and egg yolk had been added 48 h previously. Ten cultures were prepared in this manner and pooled 10 d later. The copepods were removed from the medium with a net every day from the 13th to the 18th day after exposure to the dehiscing meiosporangia. Patently infected adult copepods, which varied in colour from light amber to bright orange, were separated from the other copepods and the latter were returned to the medium. Infected copepods were examined individually with a phase microscope to confirm the colour

of the gametangium(a) in the haemocoel. The intensity of the colour, that is, the amount of pigmentation, frequently varied, especially in orange plants, from one copepod to another, but generally the infected copepods could be separated into three different types; those containing (1) only bright orange gametangia; (2) only light amber gametangia; and (3) both types. After separation into types, each copepod was placed in a 35×10 mm Petri dish in 2 ml filter-sterilised filtrate from the original copepod rearing medium. At least 20 copepods of each colour type were so prepared for each experiment. In most cases, the gametangia released the gametes, which were equal in size in both colour types, within 2 h. Shortly after gamete release, the copepod died and ruptured releasing gametes into the medium. The media containing gametes were then pooled in various combinations of type and amount and tested for mosquito infectivity. Unpooled media were also tested for infectivity. This was done by combining the media containing gametes with 20 recently hatched first-instar larvae of *A. quadrimaculatus* in a 60-mm Petri dish for 24 h. The larvae were then transferred to 1 l of rearing medium and examined periodically for infection. Additionally, drops of the pooled and unpooled media were examined periodically with a phase microscope for the presence of biflagellate planonts.

The results are shown in Table 1. No mosquito infection resulted in any test where larvae were exposed to gametes of a single colour type regardless of the number of gametes used. Some infection resulted in all tests where larvae were exposed to mixtures of light amber and bright orange gametes even in cases where both colour types were from the same copepod. Interestingly, the highest infection rates per copepod were obtained in tests where gametes from copepods containing both colour types were pooled, indicating greater numbers of zygotes may be formed if gametes of opposite mating type are produced in the same copepod.

Microscopic examinations were less definitive. In pools of gametes of a single colour type, no fusion was observed on pooling although occasionally bi- and multiflagellate planonts were observed. Although biflagellate zygotes were common in the mixtures of different coloured gametes, the actual process of fusion was observed only infrequently.

These results provide strong evidence for an *Euellomyces* type of life cycle for *C. dodgei* with the differentially pigmented sexual structures, light amber and bright orange,

being of opposite mating type. A similar type of sexual pigmentation is well known in several other *Blastocladiella* with an *Euellomyces* type of life cycle. Where it occurs in the genus *Allomyces*^{9,10}, both female (unpigmented) and male (orange) gametangia occur on the same gametophytic plant, and the resultant female gametes are larger than the males. The case of *Blastocladiella variabilis*, however, more closely resembles the situation in *C. dodgei*. In *B. variabilis* Harder and Sörgel^{11,12} found that unpigmented and orange gametangia developed on separate plants and furthermore, that the resultant gametes, which were of opposite mating type, were equal in size.

The above reports provide a precedent for designating the light amber reproductive structures of *C. dodgei* as female and the bright orange as male. In summary then, *C. dodgei* seems to have an *Euellomyces* type of life cycle with sporophyte and gametophyte generations alternating between a definitive larval mosquito host and an intermediate copepod host, respectively. The meiosporangia which develop on the sporophyte release meiospores after mosquito death. The meiospores infect a copepod and each produces a separate gametophytic thallus which at maturity is converted to one or several gametangia, but releases only a single type of gamete. Female gametangia are light amber and release unpigmented gametes. Male gametangia are bright orange and release orange gametes. One or both sexes can develop in a single copepod depending on whether the infecting meiospore(s) form male or female thalli. Uniflagellate male and female gametes fuse in appropriate conditions forming a biflagellate zygote which completes the sexual cycle by infecting a mosquito larva and producing another sporophyte.

Differentially pigmented mycelia have also been observed in *Cy. vernalis* experimentally infected with *C. punctatus* and it is likely, therefore, that this species has a life cycle similar to that of *C. dodgei*.

The occurrence of differential sexual pigmentation in *Coelomomyces* should enhance efforts to evaluate the mosquito control potential of this fungal group. The orange pigmentation or lack of it can serve as a sexual marker for genetic studies directed towards selecting highly mosquito-infective strains and for investigations on the biosystematics of species of *Coelomomyces* in which this pigmentation occurs.

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Table 1 Infectivity of orange pigmented and unpigmented gametes of *C. dodgei*, alone and in combinations, for larvae of *A. quadrimaculatus*

Total no. of copepods	Colour type	No. of infected larvae out of 20 exposed in each test			Average % infection for the three tests
		Test no. 1	2	3	
1	Amber only	0	0	0	0
2	Amber only	0	0	0	0
4	Amber only	0	0	0	0
6	Amber only	0	0	0	0
1	Orange only	0	0	0	0
2	Orange only	0	0	0	0
4	Orange only	0	0	0	0
6	Orange only	0	0	0	0
2	1 Amber only + 1 orange only	7	1	4	20
4	2 Amber only + 2 orange only	8	1	6	25
6	3 Amber only + 3 orange only	9	4	7	33.3
1	Both amber and orange	4	1	2	11.6
2	Both amber and orange	4	1	3	13.3
3	Both amber and orange	11	2	5	30
6	Both amber and orange	14	3	9	43.3

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Genetic variation in multigene families

THE possibility that some important quantitative characters of higher organisms may be subject to multigene families such as that of immunoglobulin variable parts¹, poses the problem of reformulation on some of the population genetics theory. The two remarkable characteristics of multigene families as shown by Hood and others^{1–3}—the contraction and expansion of the gene number in a family and the coincidental evolution—should now

be seriously taken into account in the theoretical investigation of the evolution of higher organisms. In addition, the unit of natural selection is the family itself rather than individual mutant genes. The model of unequal crossing-over provides one of the most satisfactory explanations for the coincidental evolution¹⁻⁴. In this model, the gene lineage gradually expands or contracts within a family mainly by intrachromosomal unequal crossovers. I have shown⁵ that the dynamics of a gene lineage within a chromosome under this model and some other models is analogous to that of a mutant gene in finite populations using the diffusion model of Kimura⁶. Here I present the equilibrium properties of genetic variation of multigene families under simplifying assumptions and show that natural selection may be quite effective in keeping a large amount of variation in the family such as that of variable parts of immunoglobulins.

Let us measure genetic variation of a gene family within a chromosome by the clonality⁴ (C) defined as the sum of squares of gene lineage frequencies within a family,

$$C = \sum_i x_i^2 \quad (1)$$

where x_i is the frequency of the i th gene lineage in the chromosome. Note that the clonality is equivalent to homozygosity in the analogous population genetics model. I also use my previous model⁵ of unequal crossing-over, in which duplication and deletion of one unit by unequal crossing-over occur alternately so that the total number of units, n , in a chromosome is kept constant. Two successive crossovers (duplication and deletion) are called one cycle of the process.

Now the mean change of C by one cycle of crossing over has been shown to be⁴

$$E\{\Delta C\} = 2(1-C)/n^2 \quad (2)$$

where E stands for taking the expectation. In other words, C increases by an amount of $2(1-C)/n^2$ through one cycle of crossover. On the other hand, it decreases by mutation and there is therefore a stable equilibrium between mutation and unequal crossing-over. Let us assume that all mutations are unique and not pre-existing and let ν be the mutation rate per gene unit per generation. Let us further assume that k is the effective number of cycles of unequal crossing-over per chromosome per generation. The changes in clonality per generation by mutation (ΔC_ν) and by crossover (ΔC_k) are then expressed as

$$\Delta C_\nu = -2\nu C \quad (3)$$

$$\Delta C_k = 2k(1-C)/n^2 \quad (4)$$

Note again the analogy of this formulation with that of genetic variability in finite populations by Kimura and Crow⁷. The equilibrium value of C becomes

$$\hat{C} = k/(k+n^2\nu) \quad (5)$$

If k is proportional to n

$$\hat{C} = a/(a+n\nu) \quad (6)$$

where $a = k/n$. So far I have considered a single chromosomal family. Actually, however there are many chromosomal families in the population and ordinary crossing over occurs between the chromosomes. This would decrease the above $\hat{C}$ value. It would be expected that if the population is very large and if the inter-chromosomal exchange is frequent, the average clonality would be greatly reduced. Here if one assumes that the rate of intra-chromosomal crossover is much larger than that of inter-chromosomal crossover as has been assumed by Smith⁸, the present formulation may still apply approximately. The more general treatment is in progress. I shall show later some results of the simulation studies.

Let us now incorporate natural selection between chromo-

somes into the model. Let s be the selective disadvantage at $C = 1$ such that the fitness of a family with clonality C (W) is equal to

$$W = 1 - sC \quad (7)$$

For simplicity, the additive effect of clonality is assumed. Then the mean fitness of the population is

$$\bar{W} = 1 - s\bar{C} \quad (8)$$

and the change in fitness is proportional to the change in C , $\Delta\bar{W} = -s\Delta\bar{C}$. The fundamental theorem of natural selection by Fisher⁹ states that the rate of change of mean fitness of the population is equal to the genetic variance of fitness in the population at that time. In our case, the change of mean fitness per generation may be expressed as

$$\Delta\bar{W} = s^2 V_c / \bar{W} \quad (9)$$

where V_c is the variance of clonality among the chromosomes in a population, hence the change in mean clonality per generation due to selection becomes,

$$\Delta\bar{C}_s = -sV_c / \bar{W} \quad (10)$$

In other words, the mean clonality decreases by an amount $sV_c / \bar{W}$ each generation by this type of selection.

This variance in clonality among the chromosomes (V_c) is the crucial factor which determines the effectiveness of natural selection and represents genetic variability in the sense of population genetics. At the moment, however it seems to be difficult to evaluate V_c . In the population genetics analogy, each chromosome corresponds to subpopulations of lineages, and inter-chromosomal crossing over to migration of mutant genes. Under the simplifying assumptions that the inter-chromosomal crossover is negligibly rare compared to the intra-chromosomal crossing over and that the population size is large without selection, V_c is expected to be roughly equal to Stewart's formula¹⁰ for the variance of heterozygosity in population genetics

$$V_c \approx \frac{2\theta}{(1+\theta)^2(2+\theta)(3+\theta)} \quad (11)$$

in which $\theta = n^2\nu/k$. Such an assumption may not usually be met although my preliminary simulation studies indicate that V_c is not much smaller in magnitude than the above formula suggests relative to the mean clonality even when the assumptions are not met. Thus, by comparing the formulas (3) and (10), it can be seen that natural selection may be more effective than mutation in reducing the average clonality or in increasing genetic variation in the chromosome, since the selection coefficient (s) is much larger than the mutation rate (ν).

I present here some of the results of my preliminary simulation studies. Since the details will be published elsewhere, only the parameters and the results are given. Two cases have been studied: (1) the population size (N) = 50 (haploid), $n = 10$, $\nu = 0.004$ and $k = 1$, (2) $N = 25$, $n = 5$, $\nu = 0.002$ and $k = 1$. Each case was run with and without selection ($s = 0$ or 0.5), and with and without inter-chromosomal crossover (total of 0 or 25 recombinations per generation in case 1 and 0 or 12 recombinations in case 2). Table 1 shows the results (average values of 101–350th generations). It can be seen that the present formulation holds without inter-chromosomal crossover. The average clonality decreases considerably with inter-chromosomal crossover though selection works whether there is inter-chromosomal crossover or not.

The present model is overly simple in another respect since it assumes that selection is solely dependent on the clonality and also that the number of gene units in a family is fixed. Actually,

Table 1 Average and variance of clonality observed in simulation studies

Population size (N)	No. of units (n)	Selection coefficient (s)	Total no. of inter-chromosomal crossovers per generation	Observed mean clonality	Observed variance of clonality
50	10	0	0	0.754 (0.714)*	0.048 (0.050)†
			25	0.444	0.021
		0.5	0	0.433	0.016
			25	0.295	0.009
		0	0	0.949 (0.952)*	0.014 (0.015)†
			12	0.781	0.028
25	5	0.5	0	0.710	0.034
			12	0.604	0.029

*Calculated by the formula (5).

†Calculated by the formula (11).

the selection is not only a function of clonality but also of the combinations of mutant genes, and also the number of gene units should be a random variable and subject to natural selection. In addition, the additive selection based on clonality is too simple. In principle, the fitness of a heterozygote could be assigned by measuring the clonality in the two chromosomes, which may differ from the additive effect. The more realistic approach awaits future analyses taking these assumptions into account. The important differences of the present approach from the traditional evolutionary theory are that the mutant genes can spread via coincidental evolution and that the gene family rather than individual mutant is considered as the unit of selection. Furthermore, in the present model, the genetic variation for natural selection to operate on (V_c) is generated by mutation and crossing-over, yet the total genetic variation including within-chromosome variance ($1-C$) may be kept mainly by selection. In fact, the antibody combining sites of immunoglobulins are hypervariable (see ref. 11 for review), suggesting that the kind of selection studied here is important. If multigene families are fairly common phenomena as speculated by Hood *et al.*¹, some similar type of selection may be operating in guiding some of the important quantitative genetic variations for the adaptation and evolution of higher organisms.

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Loss of ability to kill *Fasciola hepatica* in sensitised rats

RATS infected orally with metacercariae of the liver fluke *Fasciola hepatica* develop a partial resistance to oral challenge. This resistance occurs when the sensitising flukes have reached and are living in the bile ducts; resistance also develops if the sensitising infection is terminated by anthelmintics whilst the flukes are migrating through the liver parenchyma¹⁻³. Living adult flukes can be successfully

transplanted into the peritoneal cavity of rats^{4,5}, but if the rats have been infected with *F. hepatica* metacercariae approximately 10 weeks previously, the flukes transferred into the peritoneal cavity will be killed^{2,6}. We report here that although a marked resistance to intraperitoneal challenge with adult flukes develops as early as 2 weeks after oral infection with normal metacercariae, rats which have been infected 12 months previously, and which still have living adult flukes present in their bile ducts have lost this immunity. If rats with long-standing fluke infections in their bile ducts are reinfected with metacercariae, however, the ability of the rats to kill the challenge flukes is restored.

Adult flukes, obtained aseptically from the bile ducts of donor goats, were kept individually until transfer at 37 °C in medium 199 containing 10% foetal calf serum and antibiotics⁵. Female PVG rats (Piebald Virol Glaxo) were each infected with 20 metacercariae. The faeces of rats with long-standing infections were examined for fluke eggs as previous work⁷ had shown that many rats eliminate a primary infection after 7-8 months. Control and sensitised rats received three living adult flukes intraperitoneally. The rats were killed 7 d later and flukes were recovered and examined.

The results given in Table 1 show the effect of the duration of a primary infection on subsequent challenge. Rats sensitised for 1 week only did not kill the adult

Table 1 The effect of duration of infection on subsequent challenge

Infection and duration	No. of rats	Total no. of flukes found at necropsy		
		Dead	Alive	% Dead
None (Control 1)	6	0	18	0
None (Control 2)	6	2	16	11
1 week	5	1	14	6
2 weeks	5	13	2	86
3 weeks	6	13	5	72
4 weeks	6	17	1	98
6 weeks	6	15	3	83
12 months	6(-ve)*	0	18	0
12 months	6(+ve)*	3	15	17
None (old rats) control	5	0	15	0

Each rat was challenged with three living adult flukes placed in the body cavity. These are the flukes recovered at post mortem examination and referred to in the Table. Two control groups (1 and 2) are shown as the Table summarises two experiments. The group of old control rats was included to show that surgically transferred flukes behave normally in old rats.

* (+ve) or (-ve) indicates the presence or absence of flukes in bile ducts from primary infection.

Table 2 Effect of reinfection on subsequent challenge

Age of primary infection	No. of rats	Flukes in bile ducts	Postmortem examination		% Dead
			No. of challenge flukes Dead	Alive	
12 months	7(+ve)	present	17	4	84
12 months	7(-ve)	absent	19	2	94
None (control) (old rats)	6	—	18	0	100

The first group of rats (+ve) showed fluke eggs individually in their faeces whereas the second group were negative. These two groups of rats together with uninfected controls were infected with 20 metacercariae orally and all rats challenged 3 weeks later with three adult flukes. At post mortem examination the flukes found in the bile ducts were from the primary infection. All rats showed fluke tracts on their livers.

challenge flukes placed in the body cavity, but by two weeks after sensitisation this ability to kill the challenge had fully developed and was also seen to be operative at 3, 4, and 6 weeks after sensitisation. In contrast, rats which had been infected 12 months previously had lost the ability to kill challenge flukes in spite of the continued presence of adult flukes of the primary infection in the bile ducts.

Table 2 demonstrates the re-infection with metacercariae restores the ability to kill flukes in rats infected 12 months previously, irrespective of whether the primary infection had been eliminated or not. In those rats in which adult flukes of the primary infection were still living in the bile ducts (+ve rats in Table 2), the new sensitising secondary infection of 3 weeks duration was also alive and actively migrating in the liver parenchyma, whereas the challenge flukes were killed in the body cavity.

The ability of rats to kill the adult challenge flukes was apparently dependent on a recent migration of immature flukes in the liver parenchyma. It has been suggested that the duration of liver migration (10–11 d) in mice is responsible for the stimulation of acquired immunity⁸. Rats in which flukes have reached the bile ducts progressively lose their ability to kill challenge flukes. Perhaps the adult flukes living in the bile duct either do not produce the right antigenic stimulus, or do not present it in the right situation for processing. Alternatively, the migratory phase in the liver may produce an inflammatory response of short duration acting in the body cavity. Preliminary histological observations on the cysts surrounding challenge flukes in sensitised animals showed an intense infiltration of eosinophils into the walls and lumen of the cysts.

Although it is dangerous to extrapolate from a small animal model system, it is possible that the situation seen in the field whereby ruminants such as sheep or goats with long standing fluke infections are susceptible to re-infection is similar to the phenomenon reported here.

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Microcalorimetry of isolated mammalian cells

Using microcalorimetry^{1,2}, we have measured the heat evolved by isolated cells of brown adipose tissue (BAT). This tissue generates heat in newborn and cold-adapted mammals and in hibernators during arousal from hibernation and during cold stress^{3–5}. The method can be applied generally in work with, for example, mitochondria and isolated cells and has, especially in studies of thermogenesis,

the advantage of measuring the gross calorific output of all processes in the cell. Our results, compared with others obtained by routine methods, show that it is an oversimplification to consider BAT as the major source of heat during arousal.

Noradrenaline (NA) is the physiological stimulator of thermogenesis in BAT, and the brown adipocytes responded calorigenically (Fig. 1) to stimulation with NA in accordance with the patterns known from polarographic studies. NA-induced oxygen consumption proceeds faster and for a longer time if the buffer is bubbled with 5% CO₂ in air, rather than with air⁶, and this is reflected in our measurements of calorific output (Fig. 1). Because oleate stimulates oxygen consumption in adipocytes, possibly by mimicking the action of the hormone-sensitive lipase⁷, we have investigated the calorogenic effect of 35 μ M oleate. The response of the cells paralleled those obtained by polarographic studies (not shown).

Table 1 Ratios of heat evolution to oxygen consumption

Theoretical	
NADH oxidation to water	258
Succinate oxidation to water and fumarate	151
Palmitate, stearate or oleate combustion to water and CO ₂	218
Acetate combustion to water and CO ₂	219
Palmitate oxidation to acetate and CO ₂	219
Experimental	
1 μ M NE-stimulated cells	243

The ratios are expressed as kJ per mol of oxygen. The theoretical values are calculated from¹⁴. The experimental value is calculated from the steady state 5 min after stimulation. (Ivlevs oxycalorific coefficient¹⁵ amounts to 226 kJ per mol of oxygen.)

The heat evolved by maximal NA-stimulation at 37 °C was 820 μ W per 10⁶ cells, and heat evolution continued for at least 50 min. This is five times greater (using our estimate of 10⁶ cells per g wet weight) than reported in the only other calorimetric study, made with tissue slices⁹. Based on these figures the 2 g of BAT found in a hamster weighing 100 g (with the same heat capacity as water) would be able to warm the whole hamster less than 1 °C per h (neglecting in the calculation heat loss to the surroundings). This should be contrasted to the fact that hibernating hamsters arouse, even in a cold environment, in 2 h, that is, they increase their body temperature 15 °C per h, and even though some shivering takes place in the last phase, BAT itself is clearly not able to deliver a substantial amount of the heat needed. Furthermore, our experiments were carried out at 37 °C. The heating capacity of BAT in a hibernating animal with lowered body temperature would be even less. To illustrate this we compared the NA-evoked oxygen consumption in cells at 37 °C with that at lower temperatures and found it to be decreased to 67% at 27 °C, to be less than 10% at 15 °C and to be immeasurable at 7 °C, the body temperature of hibernating hamsters.

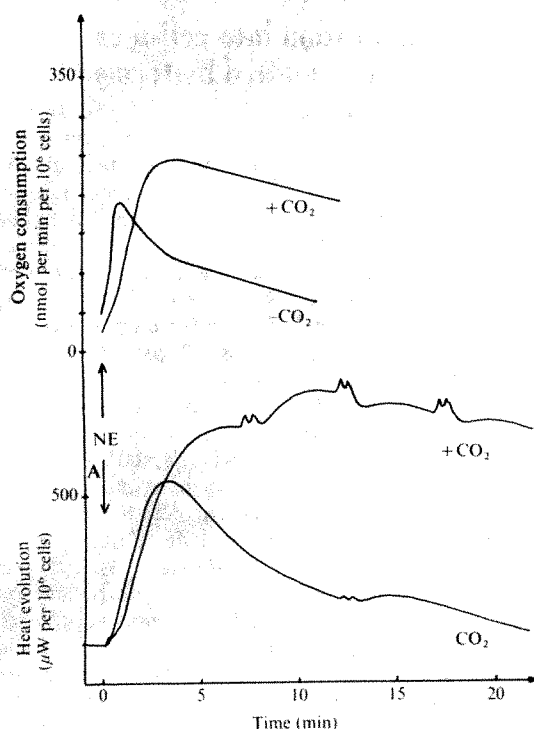


Fig. 1 Comparison between polarographic and calorimetric responses of brown adipocytes to stimulation with NA. Brown adipocytes were prepared as described before⁶ by collagenase treatment of pooled BAT from warm-adapted hamster. (In contrast to non-hibernators such as the rat, hamsters and other hibernators retain thermogenic BAT even in a warm environment.) Oxygen consumption was measured polarographically⁷ at 37 °C with a Clark oxygen electrode. Heat evolution was measured in a LKB 2107 batch microcalorimeter. The sample vessel consisted of a large compartment (4 ml) containing the cell suspension and a small compartment (1 ml) containing NA. The reference vessel contained cell suspension in the large compartment but only buffer in the other. The temperature of the thermostat was 37 °C. After equilibration, the contents of the vessels were mixed by rotation, and the difference in heat output between stimulated and unstimulated (basal respiring) cells was monitored on a potentiometric recorder. The instrument was calibrated by applying a known current through a known internal resistance. The response time of the calorimeter was in the order of tens of seconds. Control experiments showed that an unlimited supply of oxygen to the cells was ensured if the vessels were rotated every fifth minute, enabling the oxygen in the vessels to dissolve in the buffer. This was done in all experiments, giving rise to the distortions on the figures. Heat of dilution of agents has been subtracted from the curves where necessary. All cell suspensions were at 100,000 per ml in modified⁸ Krebs-Ringer phosphate buffer (unless otherwise stated the buffer was bubbled before the experiments with 5% CO₂ in air). NA was added to a final concentration of 1 μM for maximal stimulation.

Heat production is generally assumed to be proportional to oxygen consumption, but the heat produced per oxygen molecule used varies in different processes, for example, glycolysis and oxygenase reactions. Calculations¹⁰ of mitochondrial bioenergetics demonstrate a very high efficiency for formation of ATP, up to 96% energy conservation. If the ATP formed is not immediately hydrolysed in a process where the energy is not conserved, the ratio of heat: oxygen will be low. Thus estimation of heat evolution from oxygen consumption could be misleading, and we have therefore measured directly the caloric output and compared it with oxygen consumption.

Table 1 shows the calculated and measured ratios of heat evolution to oxygen consumption. The values are consistent with expectation. If CO₂ was omitted the ratio was not affected. This method does not enable us to distinguish between partial and total combustion of fatty acids^{11,12}. We find, as did Peakin¹³, that respirometry is a satisfactory method to estimate heat production.

To justify the use of isolated cells or mitochondria as model systems for the elucidation of the functions of BAT *in vivo*, we tabulated data from various laboratories as well as from different animals (Table 2). As conversion factors we used 10⁸ cells and 130 mg of mitochondrial protein per g of BAT (wet weight), obtained from morphometric studies, cytochrome oxidase measurements and protein determinations (personal communication from T. Barnard and B. Pettersson). We found a good correlation between systems. Earlier failures^{16,17} to obtain a satisfying oxidative capacity of BAT mitochondria have been due to the use of an 'unphysiological' substrate for mitochondria (2-oxo-glutarate instead of fatty acids). Only in the case of glucose uptake did we find a discordance, and we offer two explanations for this: (1) glucose permease is destroyed during cell preparation by the crude collagenase, known to have protease activity, and (2) the value from the whole animal is calculated as arteriovenous difference. The endothelium, the nerve endings and the mast cells—perhaps 10% of the tissue altogether—may utilise most of the glucose, the adipocytes using mainly fatty acids for their basal metabolism.

In newborn rabbits²⁸ and lambs²⁹ BAT accounts for most of the increase in oxygen consumption during cold-stress or infusion of NA. In cold-adapted rats it is suggested to account for 8% of the increased oxygen consumption³⁰. No direct measurements have been made on hibernators. Jansky³¹ has estimated the importance of BAT during arousal from hibernation (in bats). He measured the oxidative capacity of cytochrome aa₃ and concluded that BAT could consume 25% of the total oxygen used during arousal. This is a maximum estimate—cytochrome aa₃ is presumably not the rate-limiting enzyme in fatty acid

Table 2 Comparison between quantitative results from investigations on different organisation levels

	Heat production experimental (W)	Heat production calculated from oxygen consumption (W)	Oxygen consumption (μmol min ⁻¹)	Fatty acid release (μmol min ⁻¹)	Fatty acid uptake (nmol min ⁻¹)	Glucose uptake (nmol min ⁻¹)
Mitochondria		0.6	182 (Hamster) ^{18*} 174 (Rat) ¹⁹ 170 (Lamb) ²⁰ 226 (Guinea pig) ²¹			
Adipocytes	0.2	0.3	100 (Hamster) ⁸	8 (Hamster) ²³	400 (Hamster) ²⁵	12 (Rat) ²⁷ 120 (Hamster) ²⁵
Whole animal	See text		100 (Rabbit) ²²	4 (Rabbit) ²⁴	240 (Rabbit) ²⁶ 770 (Rabbit) ²⁴	3,000 (Rabbit) ²⁴

All values are related to 2 g of BAT as found in a newborn rabbit or an adult hamster (both 100 g). As mitochondria are traditionally examined at 23 °C, the values here have been corrected to 37 °C by a factor 2.8, obtained from experiments with hamster mitochondria (our unpublished observation and ref. 11).

*All uncoupled palmitoyl carnitine oxidation in the presence of malate.

combustion.

Our calorimetric measurements indicate that BAT has a low thermogenic capacity when compared with the heat needed to arouse a torpid hamster. Because of the general accordance between data obtained from studies *in situ* and in isolated cells, we believe that the cells elicited their full thermogenic capacity, and the opinion that BAT has thermogenesis as its primary function requires modification. Several suggestions have been made, for example, that the heat produced primarily warms the central nervous system¹ or that BAT also has an endocrine function³². During cold-stress the fat depots in BAT are emptied rapidly in both warm-adapted²⁵ and cold-adapted hamsters (our unpublished observations). As brown adipocytes are efficient in export of fatty acids^{23,24} which themselves have been shown to increase oxygen consumption in, for example, heart tissue³³, BAT may generate the main part of 'its' heat in other tissues where fatty acids liberated from BAT are combusted during cold stress. We have confirmed earlier studies³⁴ showing that in hamsters BAT does not lose any of its stored fat during arousal from hibernation. Because of these findings, and because of the relatively low calorific output reported here the direct role of BAT during arousal in the hamster must be questioned.

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Lymphocyte migration into cell-mediated immune lesions is inhibited by trypsin

THE lymph nodes and the white pulp of the spleen are major sites into which lymphocytes migrate from the blood and each organ is equally selective in that other blood cells are efficiently excluded^{1,2}. It might be expected that the same mechanism for discriminating between lymphocytes and other cells would have evolved in the spleen and lymph nodes, but the entry of lymphocytes into each of these organs can be distinguished in two ways. First, the histological appearance of the vascular endothelium across which lymphocytes migrate is dissimilar^{1,3} and second, the capacity of lymphocytes to migrate from the blood into lymph nodes is reduced by brief exposure *in vitro* to low concentrations of trypsin, but migration of the same population into the spleen is barely affected⁴. Lymphocytes are not irreversibly damaged by trypsin because they regain their capacity both *in vivo* and *in vitro*⁵ to localise in lymph nodes. The reduced numbers in lymph nodes is not a consequence of increased localisation of trypsinised lymphocytes elsewhere, as is testified by perfusing trypsinised lymphocytes through an isolated lymph node⁶ and also by measuring the numbers of trypsinised lymphocytes in the blood after intravenous injection^{6,7}. For several hours the concentration of treated cells in the blood was about 1.4 times that of control cells. This excess was attributed to the failure of trypsinised cells to enter lymph nodes⁶. We have extended these observations by comparing the capacity of trypsinised and control lymphocytes to migrate from the blood into several non-lymphoid tissues including sites of inflammation. Exposure of lymphocytes to trypsin not only exerts a differential effect on lymph node and spleen localisation, it also differentiates the mechanism by which lymphocytes migrate in small numbers into normal skin and other non-lymphoid tissues (trypsin resistant) from that responsible for the migration of increased numbers of lymphocytes into cell-mediated immune (CMI) lesions in the skin (trypsin sensitive).

To determine whether the differential effect of trypsin on lymph-node and spleen localisation is merely a quantitative effect, lymphocytes obtained by thoracic duct cannulation⁸ of AO rats were labelled *in vitro* with either ³H-uridine or ¹⁴C-uridine⁸. The ³H-labelled lymphocytes were exposed *in vitro* to graded doses of trypsin in phosphate-buffered saline (PBS), washed, combined with the ¹⁴C-labelled cells as a reference population and injected into syngeneic recipients. No reduction of spleen localisation was found with a concentration of trypsin 125 times greater than that needed to inhibit lymph-node localisation by 50% (Table 1). The standard concentration of trypsin previously used (0.02 mg ml⁻¹) (refs 4 and 6) reduced lymph-node localisation to 10% of untreated cells at 1 h after injection and increasing the dose by 5- or 25-fold had little additional effect on either the deficit in the lymph nodes or the excess in the blood. The distribution of trypsinised cells at 24 h resembled that of control cells and only at the highest concentrations was there evidence of loss of a minority of the cells. There thus seems to be a qualitative difference in the trypsin sensitivity of lymphocyte migration into the spleen and lymph nodes.

Lymphocytes also migrate from the blood into non-lymphoid tissues such as skin, albeit in much smaller numbers than into secondary lymphoid organs and the rate of migration is increased into sites of CMI (refs 9-12). We investigated whether migration into non-lymphoid tissue resembled migration into lymph nodes in being trypsin sensitive and whether trypsin treatment could distinguish between migration into normal and inflamed skin.

Lymphocytes were obtained by thoracic duct cannulation

Table 1 Effect of graded concentrations of trypsin on lymphocyte localisation in organs of recipients*Ratios of ^3H (associated with trypsinised cells) to ^{14}C (control cells)†

Time after injection	Organ	Concentration of trypsin (mg ml ⁻¹)			
		0.5	0.1	0.02	0.004
1 h	Lymph nodes	0.07	0.07	0.10	0.51
	Spleen	0.99	0.96	1.02	1.02
	Blood	1.47	1.46	1.32	1.17
	Lung	1.68	1.04	1.12	0.98
	Liver	1.35	1.22	1.01	0.86
	Gut	0.72	0.91	1.02	0.96
	Bone marrow	0.78	0.97	1.07	0.98
	Lymph nodes	0.72	0.76	0.89	0.89
	Spleen	1.18	1.06	1.05	0.92
	Blood	0.69	0.75	0.82	0.92
24 h	Lung	0.82	0.88	0.88	0.95
	Liver	1.15	0.91	1.07	1.04
	Gut	0.86	0.78	1.01	0.94
	Bone marrow	0.79	0.84	0.88	0.78

*Lymphocytes exposed to trypsin *in vitro* for 10 min.†All ratios are standardised so that $^3\text{H}/^{14}\text{C}$ ratio in the injection sample = 1.00. A supplementary group of recipients received ^3H -labelled cells (untreated) and ^{14}C -labelled cells (untreated). As expected the $^3\text{H}/^{14}\text{C}$ ratio generally fell in the range of 0.90–1.10 indicating an even distribution. In the Table, a ratio of less than 0.80 indicates a significant deficit of trypsinised cells and a ratio of more than 1.20 a significant surplus.

of inbred AS or (PVG/c×DA) F_1 rats⁸. They were radioactively labelled *in vitro* by incubation with ^{51}Cr -sodium chromate at $10 \mu\text{Ci ml}^{-1}$. The cells were suspended in medium RPMI 1640 with 10% FCS at 37°C for 1 h. After washing, half the suspension was treated with trypsin (Boehringer) at 0.02 mg ml^{-1} for 10 min. After further washing, trypsinised and control cells were injected intravenously into separate syngeneic recipients, which were killed at 0.5, 2 or 24 h after injection. A 2-ml sample of blood was taken and the entire vasculature was perfused through an aortic cannula with a large volume of PBS. The use of ^{51}Cr as a radioactive label for lymphocytes and the perfusion procedure have been found to be the most satisfactory method of measuring lymphocyte migration into non-lymphoid tissues¹³. The spleen and the six largest superficial cervical lymph nodes were removed and also samples taken of the tissues referred to in Table 2. The specimens were weighed before radioassay in a LKB γ -counter.

Two series of experiments were performed involving recipients with contact sensitivity lesions and adjuvant granulomata. Contact sensitivity was induced by painting an area of ventral abdominal skin with 0.1 ml of 5% DNCB in alcohol. Twelve days later a site on the back was painted

with a challenge dose of DNCB (0.1 ml of 0.5%). As control sites, primary application of two other contact sensitising agents, TNCB (0.1 ml of 1%) and oxazolone (0.1 ml of 0.5%), and croton oil (0.1 ml of 10%) were applied simultaneously to different areas of dorsal skin 24 h before the injection of labelled lymphocytes. In other recipients adjuvant granulomata had been produced by intradermal injection 21 d previously of 0.1 ml of Freund's complete adjuvant (Difco) emulsified with PBS.

The localisation of lymphocytes in normal skin was clearly not impaired by trypsin; radioactivity associated with trypsinised lymphocytes found in the skin soon after injection was equal to or slightly greater than radioactivity associated with untreated cells (Tables 2, 3) possibly reflecting the marginally higher numbers in the blood. It was confirmed that the migration of lymphocytes into the spleen and liver is unimpaired by trypsin treatment especially when compared with the blood level. Similarly, migration into muscle (Table 2), brain and ovary were almost equal for trypsinised and control cells suggesting that migration of lymphocytes into non-lymphoid tissues in general may be trypsin resistant. By contrast, migration into lymph nodes was reduced by trypsin to 1–2% of control values at 30 min or 2 h after injection (Table 2).

The most striking result was that trypsin treatment impaired the capacity of lymphocytes to migrate into sites of CMI in the skin (Tables 2 and 3). In the case of the DNCB lesion, the early localisation (0.5 h, 2 h) of untreated lymphocytes was increased to 1.5–2.5 times that of normal skin or sites painted with TNCB or oxazolone (Table 2). Although small, this increase was significant and occurred consistently in five other series of experiments on the migration of lymphocytes into DNCB lesions. The localisation of trypsinised cells was significantly less than that of control cells in DNCB lesions and was nearly the same as in normal skin. Thus, if it is acceptable to regard the values for normal skin as a baseline, the data indicate that very few if any trypsinised lymphocytes entered the skin as a result of DNCB challenge. The results followed the same pattern for the granuloma (Table 3) and were more emphatic: 2–3 times as many untreated lymphocytes were present in the lesions as were present in normal skin at 30 min and 2 h but equal numbers of trypsinised lymphocytes were present in the granuloma and in normal skin at each of the early intervals.

We conclude that mechanisms of selective lymphocyte migration can be placed into two categories. The first, which is trypsin resistant, operates in the spleen and non-lymphoid tissues and the second is trypsin sensitive and operates in lymph nodes and sites of CMI. Non-immune chronic inflammatory lesions have not been investigated.

It is possible that trypsin distinguishes two subsets of thoracic duct lymphocytes rather than two mechanisms of

Table 2 Effect of trypsin on ^{51}Cr -labelled lymphocyte localisation in contact sensitivity lesions
% injected activity per g tissue $\pm$ s.e.

	0.5 h <i>n</i> = 7		2 h <i>n</i> = 4		24 h <i>n</i> = 4	
	Control	Trypsin	Control	Trypsin	Control	Trypsin
Normal skin	0.029 $\pm$ 0.004*	0.032 $\pm$ 0.004†	0.013 $\pm$ 0.003	0.013 $\pm$ 0.002	0.014 $\pm$ 0.001	0.011 $\pm$ 0.001
DNCB ⁸ (<i>n</i> = 16)	0.044 $\pm$ 0.003†	0.034 $\pm$ 0.002§	0.032 $\pm$ 0.011	0.020 $\pm$ 0.003	0.052 $\pm$ 0.015	0.041 $\pm$ 0.010
Croton oil	0.028 $\pm$ 0.005	0.027 $\pm$ 0.005	0.018 $\pm$ 0.004	0.018 $\pm$ 0.004	0.021 $\pm$ 0.003	0.021 $\pm$ 0.004
TNCB	0.020 $\pm$ 0.001	0.020 $\pm$ 0.003	0.012 $\pm$ 0.002	0.011 $\pm$ 0.001	0.012 $\pm$ 0.002	0.012 $\pm$ 0.002
Oxazolone	0.024 $\pm$ 0.005	0.023 $\pm$ 0.002	0.012 $\pm$ 0.002	0.014 $\pm$ 0.003	0.014 $\pm$ 0.002	0.013 $\pm$ 0.001
Blood leukocytes	0.41 $\pm$ 0.09	0.51 $\pm$ 0.08	0.07 $\pm$ 0.007	0.15 $\pm$ 0.03	0.14 $\pm$ 0.01	0.14 $\pm$ 0.01
Lymph nodes	11.3 $\pm$ 3.11	0.11 $\pm$ 0.01	16.8 $\pm$ 4.4	0.35 $\pm$ 0.08	58.2 $\pm$ 8.7	51.6 $\pm$ 7.6
Spleen	82.1 $\pm$ 5.6	104.7 $\pm$ 7.9	94.6 $\pm$ 7.9	139.6 $\pm$ 8.2	50.8 $\pm$ 2.7	52.5 $\pm$ 2.1
Liver	2.1 $\pm$ 0.33	2.8 $\pm$ 0.45	1.2 $\pm$ 0.18	1.5 $\pm$ 0.07	1.2 $\pm$ 0.05	1.3 $\pm$ 0.05
Muscle	0.013 $\pm$ 0.002	0.019 $\pm$ 0.002	0.006 $\pm$ 0.001	0.008 $\pm$ 0.002	0.006 $\pm$ 0.001	0.004 $\pm$ 0.001
Bone marrow	3.5 $\pm$ 0.35	2.5 $\pm$ 0.33	3.7 $\pm$ 0.25	2.5 $\pm$ 0.05	0.88 $\pm$ 0.11	0.69 $\pm$ 0.12

*Against †, $P < 0.05$; † against §, $P < 0.02$; ‡ against §, not significant by the Mann-Whitney U test.

Table 3 Effect of trypsin on ^{51}Cr -labelled lymphocyte localisation in adjuvant granuloma
% injected activity per g $\pm$ s.e.

	0.5 h (n = 2)		2 h (n = 2)		24 h (n = 2)	
	Control	Trypsin	Control	Trypsin	Control	Trypsin
Normal skin	0.027 $\pm$ 0.006	0.037 $\pm$ 0.001	0.021 $\pm$ 0.005	0.025 $\pm$ 0.002	0.020 $\pm$ 0.002	0.016 $\pm$ 0.002
Granuloma (n = 8)	0.069 $\pm$ 0.004*	0.038 $\pm$ 0.002†	0.039 $\pm$ 0.002‡	0.021 $\pm$ 0.001§	0.27 $\pm$ 0.02	0.10 $\pm$ 0.01
Blood leukocytes	0.42 $\pm$ 0.19	0.69 $\pm$ 0.08	0.10 $\pm$ 0.02	0.12 $\pm$ 0.003	0.21 $\pm$ 0.015	0.10 $\pm$ 0.034

Other tissues similar to Table 2.

*Against †, $P < 0.002$; ‡ against §, $P < 0.002$ by the Mann-Whitney U test.

selective migration which are properties of different endothelia. In particular, could the inhibitory effect of trypsin on migration into sites of CMI be exerted solely on large lymphocytes which are known to migrate especially well into sites of inflammation¹⁰⁻¹²? The present results do not completely exclude this but we feel it is likely that trypsin does impair small lymphocyte migration into CMI lesions for two reasons. First, small lymphocytes do migrate into CMI lesions in increased numbers as has been supported by cannulation of the lymphatic draining an adjuvant granuloma in sheep⁹ and selectively labelling small lymphocytes with ^3H -thymidine *in vivo* and detecting an increased localisation in DNCB lesions (G.H.R., unpublished). The effect of trypsin in preventing the increased localisation in CMI lesions was complete within the limits of detection (Tables 2, 3) and so the migration of both small and large lymphocytes was probably impaired. Second, large lymphocytes not only migrate preferentially into inflamed tissues but seem to be superior in migrating into non-lymphoid tissues in physiological conditions. This was suggested by injecting alternately labelled large and small lymphocytes and examining the cells migrating into normal rat skin¹³ and non-inflamed peritoneal cavity (O. Braendstrup and O. Werdelin, unpublished). In conclusion, although it is possible that trypsin may affect lymphocyte subsets differentially, it seems improbable that this factor could wholly explain the present results; at least some lymphocytes are capable of leaving the blood by either of two mechanisms.

In an adjuvant granuloma in sheep, the increased flux of lymphocytes was associated with a change in the appearance of the endothelium of small venules, which came to resemble the morphologically distinct high-walled endothelium in lymph nodes⁹. This is consistent with the notion that a granuloma accepts an increased influx of lymphocytes by the same mechanism as lymph nodes accept more lymphocytes from the blood than do non-lymphoid tissues. Of the two examples of a trypsin-sensitive mechanism of selective lymphocyte migration, the endothelial change in CMI may have appeared earlier in evolution since histologically well developed lymph nodes are confined to mammals. Possibly lymph nodes developed when the endothelium of small vessels in certain regions displayed as a permanent feature an adaptation which was previously unique to sites of chronic inflammation. Other features of lymph node organisation may have ensued as a result of the vascular change.

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Steroid-nucleosides possible novel agents for cancer chemotherapy

THE major classes of anti-cancer chemotherapeutic drugs include alkylating agents, 'anti-metabolites of nucleic acids, steroid hormones and antibiotics'. Because of the lack of specificity of anti-tumour agents, attempts have been made to increase their specificity and cell-penetrating properties by coupling them to steroid hormones^{2,3}. In particular, steroidal alkylating agents exhibit carcinostatic properties if used in combination with other modes of therapy⁴. We have prepared a novel class of agents by coupling steroids with naturally occurring purines and pyrimidines by way of a C-N linkage. The structures are unique in that they may be regarded as analogues both of steroid hormones and of 'nucleosides' in which the sugar has been replaced. Accordingly, we may expect their steroid moiety to provide target specificity, while their nucleoside characteristics may result in interference at the DNA level to give the desired anti-tumour activity. Preliminary studies on the effect of steroid-nucleosides on the lifespan of tumour-bearing animals show that selectively coupled products exhibit anti-tumour activity.

The chemical combination of cholesterol, pregnenolone, progesterone and cortisone with various nucleoside bases and imidazole-derived heterocyclic compounds was accomplished by adapting general methods used in nucleoside synthesis⁵. Briefly, the appropriate bromosteroid was reacted with the persilylated heterocyclic in the presence of an HgO/HgBr_2 catalyst to give a C-N linkage. (Details of these preparations will be presented elsewhere.)

A number of our newly-synthesised steroid-nucleosides were tested for acute toxicity on CD-1 outbred albino female mice at 100 and 200 mg kg⁻¹ single dose levels (intra-peritoneally, 0.2 ml sesame oil). None of these compounds proved to be toxic at these levels, with the exception of the 3 α -imidazole derivative of pregnenolone. This compound induced convulsions within 1 h at the lower concentration, and death within 12 h at the higher dose. A series of thymine and uracil derivatives were tested for anti-tumour activities on Fisher 344/CRBL female rats bearing the 13762 mammary adenocarcinoma. This tumour is sensitive to oestrogens and androgens. Progesterone does not significantly inhibit tumour growth⁶, however, even at

dose levels 15 times higher than those used in our screening (D. J. Taylor, personal communication). Accordingly, it is unlikely that any anti-tumour effects of our steroid-nucleosides could result from breakdown of the drugs to give the free steroid. The compounds selected for testing included the N_1 -thymine derivatives at the 3 α -position of cholesterol (1), the 3 α - and 3 β -positions of pregnenolone (2) and (3), and the 21-position of progesterone (4); and the N_1 - and N_3 -uracil and N_1 -5-fluorouracil derivatives at the 3 α -position of pregnenolone (5), (6) and (7). The effect on the lifespan of the animals is summarised in Table 1. Control animals treated with sesame oil alone developed large tumour masses and had a mean survival of 29.1 d. In the thymine series ((1)-(4)) where the compounds differed only in the steroid moiety, the 21-progesterone analogue (4) increased the mean survival time of the host to 45.7 d, thus exhibiting marked anti-tumour activity. The remaining compounds of this series showed only minimal effect. The 3 α -pregnenolone-uracil compounds ((5)-(7)) differed in the site of attachment to the base, and although the N_1 -derivatives of uracil and of 5-fluorouracil were inactive, the N_3 -derivative of uracil (6) increased the mean survival time considerably, to 49.5 d. Treatment with 3 α -imidazolepregnenolone (8) also prolonged the survival to 40.8 d.

Table 1 Mean survival times of Fisher 344/CRBL female rats bearing the hormone-sensitive 13762 mammary adenocarcinoma on treatment with a series of steroid-nucleosides

No.	Compound	Base-steroid	C-N linkage	Survival time d ($\pm$ s.e.)	Significance P
1	Thymine-cholesterol		3 α (N_1)	29.1($\pm$ 2.8)	—
2	Thymine-pregnenolone		3 α (N_1)	34.9($\pm$ 3.0)	0.2
3	Thymine-pregnenolone		3 β (N_1)	37.3($\pm$ 3.8)	0.1
4	Thymine-progesterone		21 (N_1)	45.7($\pm$ 6.2)	0.02
5	Uracil-pregnenolone		3 α (N_1)	31.6($\pm$ 0.8)	—
6	Uracil-pregnenolone		3 α (N_3)	49.5($\pm$ 8.1)	0.01
7	5-Fluorouracil-pregnenolone		3 α (N_1)	31.2($\pm$ 1.9)	—
8	Imidazole-pregnenolone		3 α (N_1)	40.8($\pm$ 2.2)	0.001
0	Control			29.6($\pm$ 1.8)	—

Treatment was started 24 h after animals were given subcutaneous tumour grafts. The agents were administered subcutaneously in sesame oil (10 mg ml⁻¹) at a dose of 10 mg kg⁻¹ on alternate days for a total period of 18 d. Groups of 10 animals were used (except for compound (4) which involved seven animals). Of the group treated with compound (6), four animals did not develop a palpable tumour. These four survivors were not considered in the determination of the standard error and significance. The level of significance (P) was evaluated using Student's *t* test. Progesterone itself does not affect this tumour⁶.

The data suggest that greater anti-tumour activity results from an N_3 -substitution of the pyrimidine and from the use of steroids with higher hormonal activity. Most conventional drugs have undesirable side effects due to their inhibiting effect on both normal and tumour cell proliferation, and must be given at high concentration to eradicate all growing tumour cells. Our derivatives are less likely to have adverse side effects, as their decomposition products are natural steroids and nucleoside bases. Based on these encouraging results and the above considerations we are pursuing a program of selective synthesis and more detailed biological evaluation of steroid-nucleosides.

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Glucocorticoid receptors and glucocorticoid sensitivity of mitogen stimulated and unstimulated human lymphocytes

GLUCOCORTICOIDS exert widespread suppressive effects on lymphocytes of most species^{1,2}. Sensitivity to the hormones is thought to vary with the state of lymphocyte maturation and differentiation. Immature thymic lymphocytes, for example, are more sensitive than mature thymic-dependent lymphocytes^{1,3}. Furthermore, *in vitro* studies have suggested that glucocorticoid sensitivity varies with the state of mitogen or antigen-induced activation. Nowell⁴ observed that prednisolone-21-phosphate reduced the number of mitoses induced in human peripheral blood lymphocytes by phytohaemagglutinin (PHA), and noted that this inhibition required the presence of glucocorticoids during the first 24 h of exposure to PHA. When glucocorticoids were added 48 h after PHA, a time when mitoses had not yet appeared, they had no effect on the number of mitoses measured 2 d later. Tormey *et al.*⁵ extended these results to show that prednisolone-21-phosphate inhibited incorporation of radiolabelled thymidine and cytidine into PHA-stimulated lymphocytes, and found that delayed addition of steroid resulted in diminished inhibition.

These and similar observations have led to the generally accepted view, basic to the application of glucocorticoids as immunosuppressants, that glucocorticoid sensitivity varies with the state of immunologic activation of the cell. Lymphocytes are thought to be insensitive until they are stimulated by specific antigen or mitogen, which induces an early glucocorticoid-sensitive phase before resistance again supervenes with full morphologic blast transformation and mitosis^{1,6}.

Because of the central role of glucocorticoid receptors in the action of glucocorticoids on lymphoid and other tissues, we have measured simultaneously the inhibitory effects of glucocorticoids on metabolic parameters and the number of glucocorticoid receptors in cultured human lymphocytes undergoing transformation. Our results indicate that, contrary to widespread assumption, lymphocytes are sensitive to metabolic inhibition regardless of their state of activation, and that furthermore, blast transformation is associated with a striking increase in the number of receptor sites per cell.

Human lymphocytes were cultured and assayed with dexamethasone (DX) for nuclear glucocorticoid receptor sites at 37 °C as described in the legend to Fig. 1. In this experiment, following addition of concanavalin A (Con A) to cultured lymphocytes, a sixfold increase in receptor sites per cell was noted by 24 h, with maximum levels occurring before 48 h (top curve, Fig. 1). By 6 d, levels returned to

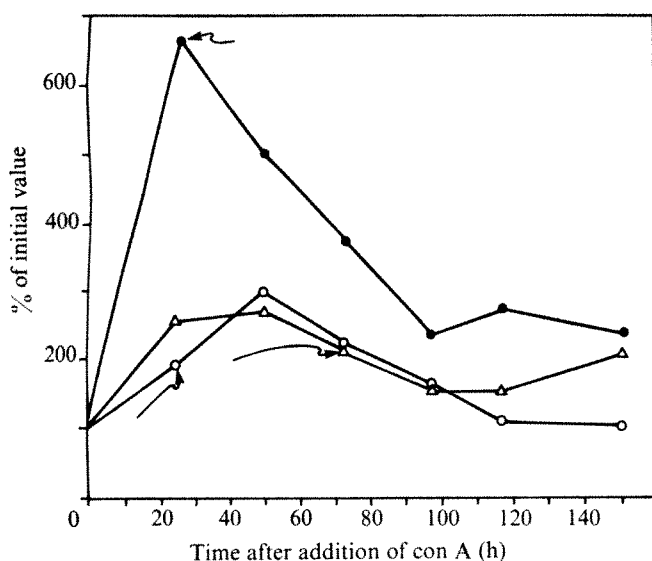


Fig. 1 Nuclear dexamethasone binding in human peripheral lymphocytes after Con A stimulation. Mononuclear cells were obtained from defibrinated fresh human blood by discontinuous density gradient separation over Ficoll-Hypaque¹⁰. Monocytes were removed by incubation on plastic for 18 h leaving a cell population consisting of 94% or more lymphocytes. Cells were cultured at 1×10^6 per ml in RPMI 1640 with 50 units per ml penicillin, $50 \mu\text{g ml}^{-1}$ streptomycin, 300 mM glutamine and 10% foetal calf serum. At time zero, $20 \mu\text{g ml}^{-1}$ con A was added. At the indicated times, aliquots containing 5×10^7 cells were removed and incubated at 37°C for 20 minutes (sufficient time to approach a steady state) with ^3H -DX (New England Nuclear; $22.6 \text{ Ci mmol}^{-1}$), at seven concentrations from $2 \times 10^{-7} \text{ M}$ to $5 \times 10^{-10} \text{ M}$. Nuclear pellets were obtained by lysis for measuring nuclear binding as described previously⁷. Results were plotted according to the method of Scatchard¹¹ to obtain estimates of the association constant and concentration of nuclear binding sites. Cell protein content was determined by the method of Lowry¹² using the supernatant after a freeze-thaw, hypotonic lysis, and cell volumes were calculated from the cell counts and the cytocrit (obtained by a micro haematocrit method) of the original cell suspension. Viability was determined from the proportion of cells excluding Trypan blue and was greater than 90% at the time of the assay. The 100% values are those for time equal to 0 h. ●, Sites per cell, 100% = 4,470. Δ, Sites per μg protein, 100% = 2.2×10^8 . ○, Moles per l cell volume, 100% = 1.5×10^{-8} .

approximately twice the initial value. Similar, but less pronounced changes were seen when results were expressed as binding sites per μg protein or per l cell volume (lower curves, Fig. 1). The exact time course of these changes was somewhat variable. In some experiments the peak number of binding sites did not occur until 72 h (for example Fig. 2). No significant changes were observed in the association constants, which averaged $1.4 \times 10^8 \text{ M}^{-1}$. Cytoplasmic receptor sites (data not shown), measured as previously described⁷, showed similar changes in number.

In separate experiments with cells from ten individuals (Table 1) we found a two-fourfold increase in DX binding sites per cell over unstimulated controls. This increase occurred regardless of the mitogenic stimulus. The controls showed no significant change in glucocorticoid binding.

Note that there was considerable variability in the receptor content of unstimulated cells. Cytoplasmic-to-nuclear translocation⁷ of the steroid receptor complex was found to take place normally in both mitogen-stimulated and control lymphocytes. When incubated with DX at 3°C , most of the binding was restricted to the cytoplasm, but after warming the cells to 37°C for 20 min, 50–80% of the complexes became nuclear bound. Cortisol, cortisone and corticosterone at 10^{-6} M competed significantly for binding of DX in both stimulated and unstimulated lymphocytes. Cortisone, testosterone, estradiol and progesterone at 10^{-6} M did not show significant competition. The time course of nuclear binding at 37°C reached a steady state after 20 min of exposure to DX for both control and con A-stimulated lymphocytes. Thus the DX-binding substances in mitogen-stimulated lymphocytes behave in all respects like glucocorticoid receptors in normal lymphocytes⁷, differing from those in the unstimulated cells only by their greater number.

The sensitivity of the Con A-stimulated lymphocytes to metabolic inhibition by DX at various times after activation is shown in Fig. 2. DX (10^{-7} M) was added after 24, 48 and 72 h of culture in the presence of con A. After 6 and 19 h exposure to DX, the incorporation of tritiated thymidine (^3H -Tdr) and tritiated uridine (^3H -Ur) was measured. The number of DX binding sites after exposure to con A was determined on separate aliquots, as described in Fig. 1. DX produced significant suppression of the incorporation of both the isotopes, but did not alter viable cell numbers. The degree of suppression of isotope incorporation did not vary significantly with time of exposure to Con A was determined on separate aliquots, as described increased fourfold during the culture period. We have also conducted these experiments with 10^{-6} , 10^{-8} and 10^{-9} M DX, with essentially the same results as with 10^{-7} M , except that suppression was dose-dependent and marginal with 10^{-9} M .

Similar studies (results not shown) were performed using unstimulated human lymphocytes placed into culture with and without DX (10^{-10} to 10^{-6} M). In five experiments there was a dose-dependent suppression of ^3H -Ur and ^{14}C -leucine incorporation of similar magnitude to the suppression shown in Fig. 2, measured after 24, 48 and 72 h of culture.

DX decreased glucose uptake by approximately the same absolute amount in both con A-stimulated and unstimulated lymphocytes (Fig. 3). The relative decrease was less for the stimulated cells, because of their higher base level of glucose uptake.

These studies demonstrate that mitogen stimulation leads to a marked increase in the number of glucocorticoid receptors per cell and that the cells remain sensitive to glucocorticoids. Our results do not necessarily contradict the earlier observations cited above. Previous investigators have generally measured the number of mitoses, number of blast transformed cells, or isotope incorporation at a fixed time after addition of mitogen with a varying time of exposure to glucocorticoids^{4,5}. These experimental approaches create an apparent loss of suppressive effect if the suppressive agent is added after the cells have begun to

Table 1 Effects of mitogenic stimuli on nuclear dexamethasone binding sites

Stimulus	No. of experiments	Dexamethasone binding sites per cell		
		Unstimulated	Stimulated	Stimulated/unstimulated
Con A	7	$3151 \pm 726^*$	$9,483 \pm 1,513^*$	$3.79 \pm 0.71^*$
PHA	1	1640	10,300	6.28
PWM	1	2490	6,750	2.71
MLC	2	$1,541; 4,070^\dagger$	$3,248; 11,160^\dagger$	$2.11; 2.71^\dagger$

Human lymphocytes were obtained and nuclear binding of DX determined as in the legend for Fig. 1. Cells were stimulated by incubation with con A $20 \mu\text{g ml}^{-1}$ for 3 d, PHA $10 \mu\text{g ml}^{-1}$ for 3 d and PWM (Pokeweed mitogen, GIBCO) for 5 d. Lymphocytes stimulated by mixed lymphocyte culture (MLC) (ref. 13) were obtained after 7 d incubation with allogeneic irradiated lymphocytes.

*Mean $\pm$ s.e.m.

† Values shown are ranges for the 1st and 2nd subject respectively.

proliferate in an exponential fashion. To accurately determine sensitivity, cells must be allowed to pass through blast transformation and equal numbers of cells, at the same stage of activation, must be compared with and without the suppressive agent. As we have shown using this experimental approach, human lymphocytes are equally sensitive to glucocorticoid suppression at all stages of activation, despite large increases in receptor number.

The increase in receptor number which follows mitogen stimulation may reflect variations in receptor number with phases of the cell cycle, a degree of synchronisation having been achieved by the sudden stimulation with mitogen. Cidlowski and Michaels⁸ have found that in synchronised HeLa cell cultures there is a doubling of receptor number per cell as the cells pass through late G₁, followed by return to the original number after mitosis.

The finding of an increased number of receptors in blast transformed cells, and the fact that receptor number does not relate directly to the level of glucocorticoid effect, raises practical considerations in the evaluation of glucocorticoid receptors and sensitivity of malignant lymphocytes. Lippman *et al.*⁹ have reported that lymphoblasts from patients with acute lymphoblastic leukaemia have higher levels of receptors than normal peripheral lymphocytes.

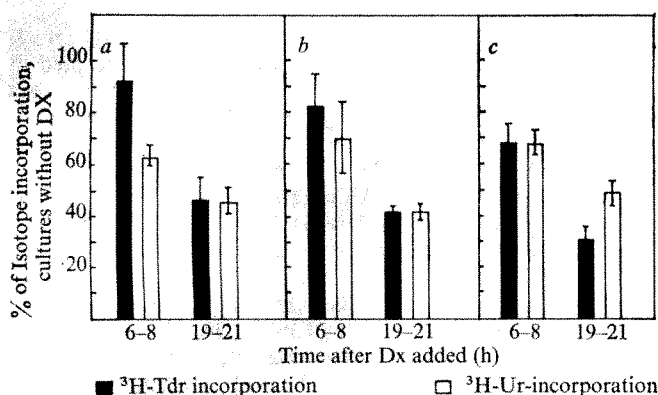


Fig. 2 Relationships between con A-induced blastogenesis, glucocorticoid receptor sites and DX suppression of tritiated thymidine and tritiated uridine incorporation. Lymphocytes (1×10^6 cells per ml) were cultured in RPMI 1640 medium, 10% autologous plasma, penicillin G, 50 units per ml, and streptomycin $50 \mu\text{g ml}^{-1}$. Con A ($20 \mu\text{g ml}^{-1}$) was added as a mitogenic stimulus. Cells were collected after 24, 48 and 72 h, and washed twice with Hanks balanced salt solution. The number of glucocorticoid receptor sites was determined as described in Fig. 1. The percentage of lymphoblastoid cells was determined on Wright-Geimsa stained Cytospin (Shandon-Southern Instruments) preparations. Cells were scored as blastoid if they were large with light staining nuclei, prominent nucleoli, and increased basophilia of the cytoplasm. Five hundred cells were counted. Aliquots of these cells (1×10^5 in triplicate) were placed into microtest-II plates (Falcon) with and without DX (10^{-7} M). Tritiated thymidine ($2 \mu\text{Ci ml}^{-1}$; 1.9 Ci mmol^{-1}) or tritiated uridine ($2 \mu\text{Ci ml}^{-1}$; 1.9 Ci mmol^{-1}) were added to the cultures from 6 to 8 and 19 to 21 h after addition of DX. Cultures were harvested on to glass fibre filters by means of a multiple automated sample harvester, washed with distilled water¹³, dried and counted in a liquid scintillation counter. Results are expressed as a percentage of isotope incorporation of cultures without dexamethasone. Brackets represent one standard deviation of per cent control. Viable cell numbers were determined by Trypan blue dye exclusion and Model B Coulter Counter before addition of DX and at the time of cell harvest. There was no significant change in cell number. The 100% values expressed as c.p.m. of isotope incorporated ± 1 s.d. were as follows: at 24 h, 6-8-h pulse $^3\text{H-Tdr} = 44 \pm 6$, $^3\text{H-Ur} = 574 \pm 35$, 19-21-h pulse $^3\text{H-Tdr} = 1109 \pm 169$, $^3\text{H-Ur} = 905 \pm 105$; at 48 h, 6-8-h pulse $^3\text{H-Tdr} = 2674 \pm 238$, $^3\text{H-Ur} = 2511 \pm 274$, 19-21-h pulse $^3\text{H-Tdr} = 2494 \pm 334$, $^3\text{H-Ur} = 1442 \pm 39$; at 72 h, 6-8-h pulse $^3\text{H-Tdr} = 2710 \pm 131$, $^3\text{H-Ur} = 1313 \pm 72$, 19-21-h pulse $^3\text{H-Tdr} = 768 \pm 123$, $^3\text{H-Ur} = 335 \pm 27$. a, Con A present for 24 h; 44% blastoid cells; 4,190 binding sites per cell. b, Con A present 48 h; 55% blastoid cells. c, Con A present 72 h; 76% blastoid cells; 16,775 binding sites per cell. Shaded bars, $^3\text{H-Tdr}$ incorporation; open bars $^3\text{H-Ur}$ incorporation.

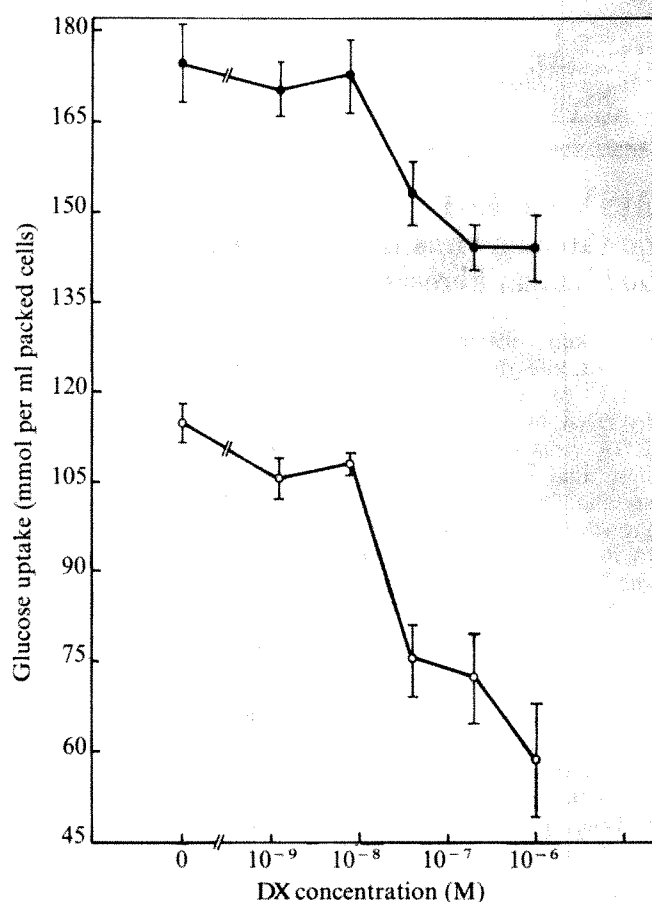


Fig. 3 Effect of DX on glucose uptake by unstimulated and con A-stimulated human peripheral lymphocytes. Lymphocytes cultured as described for Fig. 1 in the presence and absence of con A for 3 d, were washed and resuspended at 7×10^6 per ml in Krebs's Ringer bicarbonate buffer without glucose (gassed with 5% CO_2 , 95% air). At time zero, $20 \mu\text{l}$ of the cell suspension was added to $20 \mu\text{l}$ of similar medium containing 4.4 mM glucose with varying concentrations of DX and incubated in wells of V-bottom microtitre plates (Linbro) for 5 h at 37°C with 5% CO_2 in humidified air without shaking. At the end of the incubation glucose concentration in the supernatant was measured by the glucose oxidase method (Worthington). The points represent means and vertical bars represent one standard error of four replicates. $\circ$, Unstimulated lymphocytes; $\bullet$, con A-stimulated lymphocytes.

These findings have recently been extended to include lymphoblast cells from patients with Burkitt's lymphoma and poorly differentiated lymphocytic lymphoma (G. Konior, personal communication). These investigators did not explore the glucocorticoid sensitivity of the cells as it related to receptor number, but speculated that a low receptor number might correlate with glucocorticoid insensitivity. Our results suggest that in human lymphoid cells, receptor number may correlate with the proliferative stage of the cell and may not determine glucocorticoid sensitivity.

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MSA and EGF receptors on sarcoma virus transformed cells and human fibrosarcoma cells in culture

CULTURED cells transformed by murine or feline sarcoma viruses lose their ability to bind epidermal growth factor (EGF) to cell surface receptors, whereas cells transformed by DNA tumour viruses or infected with non-transforming RNA viruses show normal EGF binding¹. We demonstrate here that this sarcoma virus-dependent receptor loss is specific for EGF. The binding of a different polypeptide growth factor, multiplication stimulating activity²⁻⁴ (MSA), to its cell surface receptors was not decreased in murine and feline sarcoma virus transformed cells. In two cell lines established from human fibrosarcomas, however, MSA binding was abolished while EGF bound to the same extent as to normal human fibroblasts.

MSA is the name given to a family of polypeptides of molecular weight approximately 10,000 that is synthesised by a line of Buffalo rat liver cells in culture³⁻⁴. Like EGF and fibroblast growth factor⁵ (FGF), MSA is an efficient stimulator of cell DNA synthesis in chick embryo³, rat², 3T3 mouse⁶ and human⁷ fibroblasts in culture, and in a rat muscle cell line⁸. Specific receptors for MSA have been characterised in rat liver plasma membranes^{9,10}, and in cultures of chick embryo fibroblasts^{9,11}, adult human skin fibroblasts⁷, and a subclone of the rat liver cell line that produces MSA⁴. The binding of ¹²⁵I-labelled MSA to these receptors was inhibited only by MSA itself and by two polypeptides purified from human plasma, somatomedin A and non-suppressible insulin-like activity^{7,10,11} (NSILA-s); ¹²⁵I-MSA binding to chick embryo and human fibroblasts also was inhibited by insulin and proinsulin^{7,9,11}. Other peptide hormones and growth factors did not compete for MSA binding.

We studied MSA binding in normal rat kidney (NRK clone 2) and mink lung cells, and their sarcoma virus transformants (Table 1). The conditions of the receptor assays were those previously used to study the binding of type C

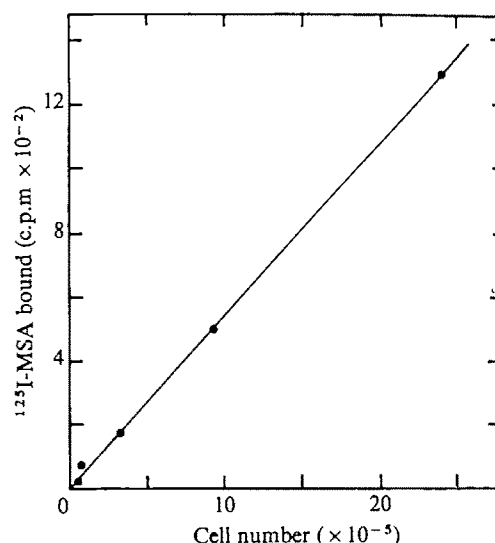


Fig. 1 ¹²⁵I-MSA binding as a function of cell number. ¹²⁵I-MSA was incubated with NRK clone 2 cells at the indicated densities per 60-mm plate. The incubations were performed as described in Table 1. The specific binding of ¹²⁵I-MSA is plotted as a function of the cell number.

viral envelope glycoprotein¹² and EGF¹ to cellular receptors, with the exception that the incubations were performed at 22 °C. Subconfluent cell monolayers were used so that both normal and transformed cells would be actively growing. MSA binding increased linearly with the number of NRK cells seeded per 60-mm Petri plate over the range 2.7 × 10⁴ to 2.4 × 10⁶ cells (Fig. 1). Although significant MSA binding can be detected with as few as 5 × 10⁴ NRK cells, most experiments were performed with 0.5–1.0 × 10⁶ cells per plate.

Normal rat and mink cells have different receptors that bind MSA or EGF selectively (Table 1, Fig. 2). The binding of ¹²⁵I-MSA was inhibited by unlabelled MSA, with 50% of the maximal inhibition by 133 ng ml⁻¹. In contrast, EGF, fibroblast growth factor, nerve growth factor, and insulin at 6.7 μg ml⁻¹ did not inhibit ¹²⁵I-MSA binding. Approximately 90% of total ¹²⁵I-MSA binding could be abolished by incubation with 3.3 μg ml⁻¹ MSA, and is referred to as specific binding. ¹²⁵I-EGF also bound specifically to normal rat and mink cells. Its binding was inhibited by EGF, but was not affected by high concentrations of MSA.

Table 1 ¹²⁵I-EGF and ¹²⁵I-MSA binding to normal and transformed cells

¹²⁵ I-labelled	Ligand	Unlabelled	Normal rat kidney cells		Normal mink lung cells		FeSV transformed
			Control	K-MSV transformed	Control	K-MSV transformed	
EGF	None		1,970	120	9,430	320	440
EGF	EGF		170	180	190	190	150
EGF	MSA		1,400	120	9,700	260	420
MSA	None		1,445	930	1,023	1,090	1,145
MSA	MSA		120	135	131	175	110
MSA	EGF		1,945	925	1,156	1,010	1,215

¹²⁵I-EGF and ¹²⁵I-MSA binding to normal cells and cells transformed by Kirsten murine sarcoma virus (K-MSV) and feline sarcoma virus (FeSV). EGF was prepared from male mouse submaxillary glands by acid extraction followed by gel filtration and DEAE-cellulose chromatography¹³. MSA was purified from the serum-free conditioned media obtained from a rat liver cell line (BRL3A) in culture^{4,5}. Purification procedures included ion exchange chromatography through Dowex-50, gel filtration chromatography on Sephadex G-50 at acid pH, and preparative acrylamide electrophoresis⁴. The iodination of EGF¹⁰ and MSA^{9,11,13} binding assay procedures and cell designations have been previously described⁴. Cells were plated in 60-mm plastic Petri dishes (Falcon) in Dulbecco's modified Eagle's medium (DMEM) containing 10% calf serum at the indicated densities: control normal rat kidney, NRK clone 2 (5 × 10⁶ cells per dish); K-MSV transformed NRK (5 × 10⁶ cells per dish); control normal mink lung cell (7 × 10⁶ cells per dish); K-MSV transformed mink lung cell (6 × 10⁶ cells per dish); and FeSV transformed mink cell (6 × 10⁶ cells per dish). After 18 h the plates were washed twice with 1.5 ml of binding buffer (DMEM containing 1 mg ml⁻¹ bovine serum albumin and 50 mM N,N-bis-(2-hydroxyethyl)-2-aminoethane-sulphonic acid (BES), pH 6.8). Binding reactions were initiated by the addition of 1.5 ml of binding buffer containing either 3 ng of ¹²⁵I-EGF (120,000 c.p.m.) or 0.4 ng of ¹²⁵I-MSA (34,200 c.p.m.) with or without 5 μg of unlabelled EGF or MSA. The dishes were incubated at 22 °C for 90 min. At the end of the incubation, the media was removed and the cell monolayers were washed four times with cold binding buffer to remove the unbound ¹²⁵I-peptides. The amount of ¹²⁵I-peptide bound to the cells was measured after lysing the cells. The radioactivity present in the lysate⁴ was determined using a Beckman LS-250 liquid scintillation counter at 60% efficiency.

Transformation of rat and mink cells with either murine or feline sarcoma viruses abolished approximately 90% of the specific EGF binding, but did not significantly reduce MSA binding (Table 1). Similar results were obtained when the binding of MSA and EGF to normal mink lung cells and mink lung cells transformed by murine sarcoma virus was compared after incubation for different times ranging from 15–180 min (Figs 3a, b). These results confirm that sarcoma virus transformation selectively perturbs the EGF receptor system.

We next asked whether the ability to bind MSA might be lost in other types of transformed cells. Mouse and rat clones transformed by the DNA viruses SV40, polyoma, and adenovirus, as well as a series of chemical carcinogen-transformed mouse clones were examined. In 17 of 18 clones tested MSA was bound to a similar extent in the transformed and parental cell lines. One clone of polyoma-transformed 3T3, PY-3T3-4A (ref. 13), has consistently shown low levels of MSA binding (10–20% of the control 3T3 cells), but three other independently isolated polyoma-transformed 3T3 clones showed normal levels of MSA binding. Transformation of cells in culture, then, is apparently not associated with a specific alteration of MSA receptors.

In striking contrast is the selective loss of MSA binding to two cell lines that have been established from human fibrosarcomas (Figs 3c,d). These lines, 8387 (ref. 14) and HT1080 (ref. 15) have been previously characterised. Line 8387 was derived from a 25-yr-old female with fibrosarcoma of the leg; HT1080 was from a 35-yr-old male with a fibrosarcoma of the acetabulum. Less than 5% as much ^{125}I -MSA was bound by the 8387 and HT1080 fibrosarcoma cell lines than by foetal and newborn fibroblasts. In contrast, ^{125}I -EGF bound to comparable extents and with the same kinetics to the same fibrosarcoma cells and to embryonic and newborn human fibroblasts. The low binding of ^{125}I -MSA to the fibrosarcoma cells did not result from an altered kinetics of binding (Figs 3c, d). Neither did it seem to result from degradation of ^{125}I -MSA by the fibrosarcoma cells. Pre-incubation of ^{125}I -MSA with 8387 cells for 2 h

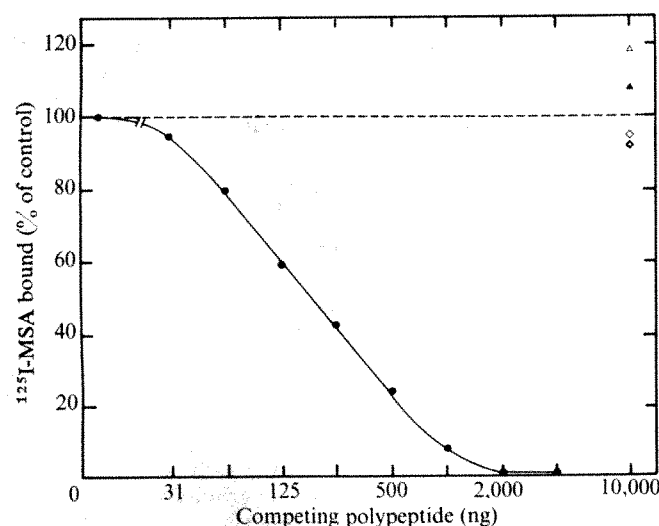


Fig. 2 Competition for ^{125}I -MSA binding by unlabelled peptides. ^{125}I -MSA (0.2 ng ml^{-1} ; $14,000 \text{ c.p.m.}$) was incubated with NRK clone 2 cells (9.5×10^5) for 90 min at 22°C in the presence of varying amounts of unlabelled peptides. MSA, $\bullet$; EGF, Δ ; nerve growth factor, $\blacktriangle$ (gift from R. Levi-Montalcini); fibroblast growth factor, $\blacklozenge$ (gift from D. Gospodarowicz); crystalline zinc porcine insulin, $\diamond$ (Lilly). Nonspecific binding (the ^{125}I -bound ligand which could not be competed by a large excess of unlabelled ligand) has been subtracted. The data presented are the mean of duplicate determinations. The % of control binding (2.1% of input radioactivity) is plotted against the log of the unlabelled competing peptide.

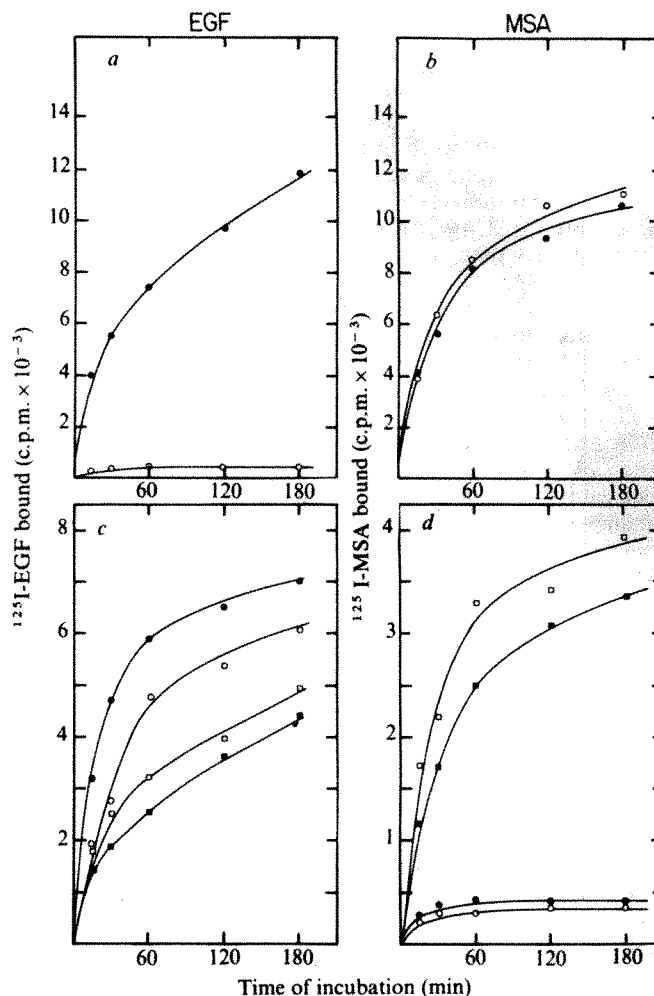


Fig. 3 Time course of association of ^{125}I -EGF (a, c) and ^{125}I -MSA (b, d) to different cell types. a, b: ^{125}I -EGF (a) or ^{125}I -MSA (b) were incubated with normal mink cells ($\bullet$, 7×10^5 cells per dish) or Kirsten sarcoma virus transformed mink cells ($\circ$, 6×10^5 cells per dish). c, d: ^{125}I -EGF (c) or ^{125}I -MSA (d) were incubated with four human lines or strains: 8387, a human fibrosarcoma line ($\bullet$, 7×10^5 cells per dish); HT1080, another human fibrosarcoma line ($\circ$, 5×10^5 cells per dish); HuF, a human foreskin fibroblast strain ($\square$, 4×10^5 cells per dish); WI38, a human embryonic lung fibroblast strain ($\blacksquare$, 4×10^5 cells per dish). The data are not corrected for counter background ($\sim 60 \text{ c.p.m.}$) nor nonspecific binding ($\sim 250 \text{ c.p.m.}$ for ^{125}I -MSA).

did not destroy the ability of the ligand recovered from the incubation medium to bind to receptor-positive cells. Similarly, fibrosarcoma cells cocultivated at fiftyfold excess (2×10^6 cells per plate) with NRK clone 2 cells (4×10^4 cells per plate) did not inhibit ^{125}I -MSA binding to the rat cells.

The lack of MSA binding to the two human fibrosarcoma cell lines is especially distinctive because MSA binds well to fibroblasts and other mesenchymal cells from humans and other species, and to cell lines established from a variety of other human tumours. MSA receptors were found in 34 out of 34 fibroblast strains from 12 different species. More than 20 normal human fibroblast strains derived from foetuses, newborns, and adults of various ages showed more than 10 times greater MSA binding than that of the fibrosarcoma lines. Further, loss of MSA receptors is not a general property of human tumour cells in culture, since other human tumour lines including chondrosarcomas, glioblastomas, and renal cell carcinomas specifically bound labelled MSA (in preparation).

Since the progenitor cells of the fibrosarcomas presumably had MSA receptors, the selective loss or unavailability of MSA receptors in the fibrosarcoma cell lines suggests the possibility that an alteration in the MSA-

receptor system may have had a role in the cellular transformation process. It is intriguing that fibrosarcomas are among the human tumour types that have been associated with clinical hypoglycaemia not attributable to elevated plasma insulin levels¹⁶. Markedly elevated levels of NSILA-s, a polypeptide with weak insulin-like activity, were identified in the serum of one patient with a fibrosarcoma and hypoglycaemia; presumably the tumour was the source of the circulating NSILA-s¹⁷. NSILA-s and MSA are closely related peptides that interact with the same receptors^{4,7,10,11}. This raises the possibility that the fibrosarcoma cell lines produce MSA-like polypeptides. The endogenous peptide production might occupy receptors, or regulate their availability¹⁸. Experiments to test for production of these factors by the fibrosarcoma lines in culture are in progress.

Thus, the binding of two polypeptide growth factors, EGF and MSA, seems to be selectively altered in different types of cell transformation. MSA binding is markedly reduced in two human fibrosarcoma cell lines, whereas EGF binding is abolished by murine and feline sarcoma virus transformation. While the precise relationship between the availability of growth factor receptors and transformation is unknown, we propose the following model for consideration. Cell growth in an organism is controlled, in part, by the particular display of specific growth factor receptors responding to hormones produced by other cells. In the normal situation, those cells that produce a specific growth factor do not also respond to it. But, the inappropriate production by a target cell of an active hormone that it also has receptors for may be sufficient to serve as a stimulus to cell division. Persistent uncontrolled production of the growth factor may then serve as a continuous endogenous stimulus and lead to inappropriate cell growth.

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Simian virus 40 facilitates multiplication of replication defective mutants of polyoma virus in BALB/3T3 mouse cells

STUDIES on conditionally lethal mutants of polyoma virus or simian virus 40 (SV40) should provide information on viral gene functions in infected cells. Temperature-sensitive mutants of polyoma virus^{1–4} and SV40 (refs 5–8) and a host range mutant of polyoma virus^{9,10} have already been isolated. But, the functional relationship between the viral genes of polyoma virus and SV40 has not yet been studied. To examine the relationship between the viral gene functions of polyoma virus and SV40, and changes of the cellular regulation system in cells transformed by SV40, we attempted to isolate new host range mutants of polyoma virus which could replicate in mouse cells transformed by SV40 but not in normal mouse cells. We report here the isolation of two host range mutants of this type. Productive infection of these mutants in A31-714 cells¹¹ (subclone of mouse BALB/3T3), which are restrictive for these mutants, is facilitated by co-infection with SV40.

Host range mutants were isolated from ultraviolet-irradiated polyoma virus (strain: LP147-2). A sample of 2 ml of fluid containing polyoma virus was put into a Petri dish (40 mm in diameter) and irradiated for 3 min under a Toshiba germicidal lamp (15 W×2) from a distance of 15 cm with gentle shaking. In these conditions, the plaque-forming ability of the polyoma virus was reduced to 10⁻³ of the initial value. After irradiation, appropriate dilutions of the sample were plated on subconfluent monolayers of SA31 cells (A31-714 cells transformed by SV40 with the following properties; (1) SV40 T-antigen positive, (2) virus-free, (3) SV40 inducible by cell fusion with CV-1 cells by UV-HVJ, and (4) capable of use in plaque assay of polyoma virus). After 7–8 d single plaques were picked up and each was tested for its ability to produce plaques on confluent monolayers of A31 cells.

Two of 576 plaques tested failed to produce distinct plaques on A31 cells. The mutants obtained by three further sequential plaque purifications on SA31 were named HR-86 and HR-101.

Table 1 shows the abilities of mutant HR-101 to form plaques on monolayers of A31-714 cells and SA31 cells. The plaque forming ability of the wild type was the same on both indicator cells, whereas that of HR-101 was 10⁻²

Table 1 Plaque-forming abilities of HR-101 in A31-714 cells and SA31 cells

Type of virus	Type of cells	PFU per ml
HR-101	A31-714	< 10 ⁴
HR-101	SA31	3.1 × 10 ⁶
WT	A31-714	2.1 × 10 ⁶
WT	SA31	2.0 × 10 ⁶

Plaques were counted 8 d after virus infection. PFU, plaque-forming units.

or less on A31-714 cells than on SA31 cells. Cytopathogenic effects were seen, however, when low dilutions of HR-101 samples were inoculated on to monolayers of A31-714 cells.

To see whether the reduction in the plaque-forming ability of HR-101 in A31-714 cells was due to decrease in virus yield, the replications of the mutant in A31-714 cells and in SA31 cells were compared.

A31-714 cells and SA31 cells were both infected with HR-101 and 6 h later uninfected virus was washed out and production of virus was followed at 24-h intervals.

When HR-101 was inoculated on to A31-714 cells, it did not show appreciable multiplication, but when it was inoculated on to SA31 cells it multiplied: multiplication

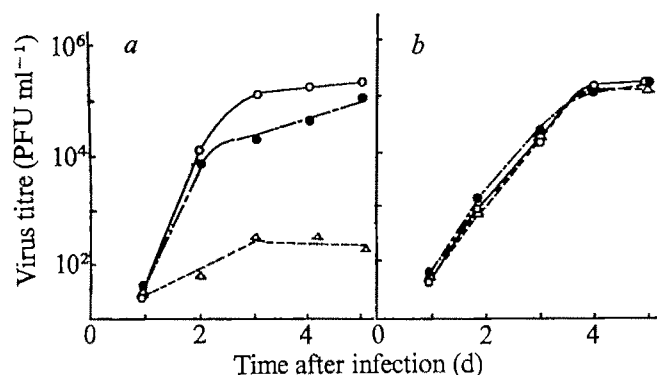


Fig. 1 Cultures of A31-714 and SA31 inoculated with HR-101, Wt, HR-101 plus SV40 (777 strain) or WT plus SV40. The multiplicities of infection of HR-101 and WT were 0.03 and that of SV40 was 60. After adsorption at 37 °C for 6 h, cultures were washed three times with medium, covered with 5 ml of Eagle's MEM medium containing 10% calf serum and incubated at 37 °C at 24 h intervals, cultures were collected and stored frozen. Cells and media were sonicated and their virus titres were assayed by plaque formation on cultures of SA31 cells. Growth of HR-101 (a) and WT (b) in SA31 cells (○), in A31-714 cells without SV40 (△) and in A31-714 cells with SV40 (●).

was detectable after 2 d and reached a maximum after 3–4 d (Fig. 1a). In contrast, the wild type multiplied equally well in A31-714 cells and SA31 cells (Fig. 1b).

To determine whether the change in susceptibility of A31-714 cells to infection by the mutant was caused by SV40, the helper function of SV40 in multiplication of the mutant in A31-714 cells was also examined. A31-714 cells were co-infected with HR-101 (multiplicity of infection (m.o.i.), 0.03) and SV40 (m.o.i., 60) and the yields of HR-101 were followed by the method described above.

The yield of HR-101 in A31 cells was enhanced approximately 60-fold by co-infection with SV40 (Fig. 1a).

When SV40 samples were treated with anti-SV40 rabbit serum, their ability to enhance the replication of HR-101 disappeared completely (Table 2). The viral characters of HR-86 were similar to those of HR-101 described above.

In preliminary experiments, cells in primary cultures from embryos of a BALB/c mouse also showed low susceptibility to host range mutants and co-infected SV40 enhanced the growth of these mutants in the primary cells (data not shown).

These results suggest that SV40 converts the susceptibility of mouse cells to infection with these mutants from a restrictive to a non-restrictive state. Although the role of SV40 in this conversion of cellular susceptibility has not yet been elucidated, the restrictive point to the infection of HR-101 in A31-714 cells may be considered not before the penetrat-

Table 2 Effect of anti-SV40 serum on the helper function of SV40

Type of infection	Type of cells	Virus titre per ml*	Enhancement ratio
HR-101	A31-714	7.8×10^3	1.0
HR-101+SV40	A31-714	4.5×10^4	57.7
HR-101+(SV40)†	A31-714	2.8×10^3	0.4
HR-101	SA31	1.0×10^5	128.2

Cultures of A31-714 and SA31 containing 3.2×10^5 cells per 4-cm plastic dish were inoculated with HR-101 at a multiplicity of 0.03. Some cultures of A31-714 were inoculated with both HR-101 and SV40 (m.o.i., 10) or HR-101 and SV40 which had been treated with anti-SV40 serum. After adsorption at 37 °C for 6 h, cultures were washed three times with medium and covered with 5 ml of Eagle's MEM medium containing 10% calf serum. These cultures were collected 72 h later. The cells and media were sonicated and virus infectivity in the lysates was assayed by plaque formation on cultures of SA31 cells.

*72 h post-infection.

†Treated with anti-SV40 serum.

ing step but after the uncoating step, because DNA from HR-101 was also considerably less infectious to A31-714 cells than to SA31 cells (Table 3). The defect in virus growth of the mutants may be helped either by the direct action of a SV40 viral gene product(s) present in both SV40-transformed cells and mouse cells abortively infected with SV40, or by the indirect action in which a SV40 genome in these cells alters the expression of cellular genes. In any case, an 'early' event(s) of SV40 infection may be concerned with change in susceptibility to these host range mutants because SV40 did not produce 'late' gene products in mouse cells on either infection or transformation^{12,13}.

Table 3 Virus yields after infection with viral DNA

DNA	DNase treatment (20 µg ml ⁻¹)	Yield (PFU per culture) A31	Yield (PFU per culture) SA31	Ratio SA31 : A31
Wt	—	1.5×10^3	1.8×10^3	1.2
	+	< 10	< 10	—
HR-101	—	5.0×10^4	9.0×10^3	180.0
	+	< 10	< 10	—

A confluent monolayer of A31-714 cells or SA31 cells in a 6-cm Petri dish was washed once with 5 ml of phosphate-buffered saline and then infected with 0.1 ml of a mixture containing wild-type DNA (7.2×10^3 PFU: assayed on SA31 cells) or HR-DNA (2.8×10^3 PFU: assayed on SA31 cells), DEAE-dextran (500 µg ml⁻¹) and Tris-HCl (pH 7.5, 0.05 M). After incubation at room temperature for 30 min, the cells were washed once and then 5 ml of growth medium (MEM + 10% calf serum) were added. After 3 d at 37 °C, cells and fluids were collected, sonicated and assayed on SA31 cells to determine virus yield.

Further experiments on the characteristics of host range mutants and on the relation between the gene functions of polyoma virus and SV40 are in progress.

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Extraneural localisation of herpes simplex virus in latently infected guinea pigs

CHRONIC latent herpes simplex virus (HSV) infections are associated with tissues of the nervous system in experimentally infected mice and rabbits^{1,2} as well as in humans^{3–5}. Cook and Stevens demonstrated, that, after inoculation into the skin, HSV migrates via the subserving nerves to the corresponding sensory ganglia⁶. The virus establishes there a latent infection which can be detected by methods of *in vitro* cultivation of the ganglia^{1–3}. A consideration of the data obtained so far on the behaviour of latent HSV in mice, rabbits and men led Cook and Stevens as well as Baringer to the postulate, that latent HSV infections were restricted to tissues of the nervous system^{7,8}. The failures to detect HSV in the skin during latent infections are con-

Table 1 Recovery of HSV type 2 during the chronic latent infection

Animal	Day after infection	Footpad	Sciatic nerve	Virus recovered from Dorsal root ganglia (L ₄ -S ₃)	Lumbosacral spinal cord	Number of recurrent eruptions	Day after infection when last recurrence developed
261	97	+	+	+	ND	None	—
885	97	+	+	+	ND	1	41
298	97	+	—	—	ND	None	—
274	133	+	—	+	—	2	73
839	136	+	—	+	—	2	80
569	136	+	—	+	—	4	104
272	143	+	—	+	—	None	—
241	143	—	+	—	—	None	—
775	194	+	—	—	+	2	125
88	194	+	—	+	—	3	38
230	208	+	+	—	+	1	38
757	208	+	?	—	+	1	73
799	210	+	+	—	ND	4	110
883	210	+	+	+	ND	2	95
603	210	+	+	—	ND	2	58

ND, not done. ?, Cultures contaminated.

sistent with this hypothesis^{9,10}. Recurrent HSV infections are thought to arise by activation of the latent infection within the ganglia and the subsequent migration of the reactivated virus into the skin via the corresponding nerve fibres^{8,11}. I have described recently experimental infections of guinea pigs with HSV type 2, which led to chronic latent infections of the animals with frequent spontaneously recurring lesions at the sites of initial inoculation^{12,13}. In this report I present evidence that during the latent phase of the infection HSV can be recovered not only from nervous tissues but also from the skin at the site of the primary infection.

Fifteen albino guinea pigs were inoculated with 10⁴ plaque-forming units of HSV type 2, strain 72, subcutaneously into the left hind footpad. They were observed for clinical signs of primary and recurrent herpetic lesions three times a week. The animals were sacrificed between 3 and 7 months after infection at times when they did not show any clinical sign of recurrent infection. Pertinent tissues were taken from exsanguinated animals and assayed for latent virus by explantation on monolayers of primary rabbit kidney (PRK) cells as described previously¹¹. All isolates were passaged once on PRK cells and identified as HSV by neutralisation with an anti-HSV hyperimmune serum, prepared in rabbits.

The data (Table 1) show, that all 15 animals harboured latent HSV, irrespective of whether they had developed previous recurrent infections or not. Surprisingly, however, from 14 of the animals virus was isolated from the skin of the initially infected footpad, whereas only eight animals were shown to harbour virus in their lumbosacral dorsal root ganglia and three animals in the lumbosacral part of the spinal cord. In addition, HSV was recovered from the sciatic nerve trunks of seven animals.

To test whether chronic latent infections of guinea pigs could be established with HSV type 1 as well and if so, where the virus would be located, a group of animals was similarly infected with HSV type 1, strain McIntyre. This strain does not induce recurrent infections in guinea pigs (unpublished). Chronic latent infection of the skin of the footpad was detected in six out of eight animals (Table 2).

None of the eight guinea pigs, however, could be shown to harbour latent virus in their nervous system.

The surprising finding that HSV could be recovered preferentially from the skin of the footpad during the clinically quiescent phase of the infection offers two alternative interpretations. First, in contrast to the well documented restriction of latent HSV infections to tissues of the nervous system in mice and rabbits, guinea pigs may harbour latent HSV type 2 preferentially and latent HSV type 1 exclusively in the skin at the site of the primary inoculation. Alternatively, HSV infections of guinea pigs may be no exceptions to the rule that latent virus resides exclusively in nervous tissues. Such latent virus may, however, become frequently or even consistently reactivated in the ganglia to spread via peripheral nerves into the skin, although overt clinical disease may be only rarely induced. The latently infected ganglia may harbour only small amounts of virus, frequently escaping detection in our assay, whereas the skin, and in some animals also the nerve trunks, may contain enough reactivated virus to be more easily recovered.

The second explanation seems more likely as it is essentially consistent with data, obtained in other species. In addition, it could support a hypothesis on the pathogenesis of recurrent herpes infections, suggested recently by Hill and Blyth¹⁴. According to this theory, virus is frequently activated in ganglia, travels to the skin, and induces micro-foci of infected epidermal cells, which are usually eliminated by the host's defence mechanisms. Visible lesions develop only under some additional alteration of the local environment. If this theory is correct, virus should be frequently detectable in the skin. The previous failures to recover virus from skin of mice and men^{9,10} using organ culture may be due to the comparatively rare spontaneous reactivation of the latent virus within the ganglia in these species.

Further experiments in neurectomised guinea pigs should help to determine which of these alternative interpretations is correct. Evidence was given in a previous report¹², that HSV travels along the sciatic nerve from skin to ganglia and vice versa. Dissection of the nerve before infections should therefore prevent the establishment of latent infection in ganglia. Consequently, in such animals neither

Table 2 Recovery of virus from animals infected with HSV type 1

Day after infection	Footpad	Virus recovered (animals positive/animals tested) from			Lumbosacral spinal cord
		Sciatic nerve	Dorsal root ganglia (L ₄ -S ₃)		
79	0/2	0/2	0/2		0/2
198	2/2	0/2	0/2		0/2
313	4/4	0/4	0/4		0/4

recurrent lesions nor latent virus in the skin of the footpad should be found, if the second explanation holds. Such experiments are currently under way.

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Relationship between cell shape and type of collagen synthesised as chondrocytes lose their cartilage phenotype in culture

WHEN chondrocytes from sternal or articular cartilage are kept in monolayer culture at low density, they eventually lose their cartilage phenotype^{1–4}. Within four passages or approximately 1 month in culture they change from a polygonal or round to a flattened, amoeboid-like shape^{5–7}, and instead of cartilage collagen (type II collagen⁸) they synthesise the genetically different type I collagen. It is not known whether there is a strict correlation between the occurrence of cell flattening and the change in collagen synthesis within individual cells. We have reported that preferentially flattened, fibroblast-like cells at the edge of cartilage colonies synthesise type I collagen, whereas round or polygonal chondrocytes generally synthesise type II collagen^{1–3}. The change is nearly complete in a culture at a time when excessive flattening is observed⁴. Using an immunofluorescence double staining technique^{9,10}, we have now found that there is no strict correlation between cell morphology and type of collagen synthesised.

We used chondrocytes from 16-day embryonic chick sternal cartilage, which were released by treatment with trypsin and collagenase^{1–3} and cultured on tissue culture plastic dishes at a density of 10^4 cells per cm^2 in Ham's F12 medium and 10% foetal calf serum. In these conditions the change from type II to type I collagen synthesis was nearly complete within 1 month.

The type of collagen synthesised by individual cells was determined at various stages of dedifferentiation by immunofluorescence using specific antibodies to type I and type II collagen⁹. This facilitated the comparison of cell shape and the type of collagen synthesised. The double staining procedure enabled us to determine whether a single cell could synthesise both types of collagen simultaneously¹⁰. During the first day of culture the chondrocytes remained almost round; staining with rabbit anti-type II collagen antibodies revealed strong fluorescence of the cell membrane and of numerous filopodia (Fig. 1a). Because of strong membrane-bound fluorescence no intracellular type II collagen or procollagen could be detected. No positive reaction with antibodies to type I collagen was obtained at this stage. After 2 d most chondrocytes had settled on the surface, flattened and retracted their filopodia. These cells showed little membrane bound fluorescence, for the nucleus appeared dark after staining with anti-type II collagen antibodies, but strong intracellular fluorescence (Fig. 1b). This was probably due to type II procollagen, the intracellular form of collagen¹¹, which seemed to be packed in

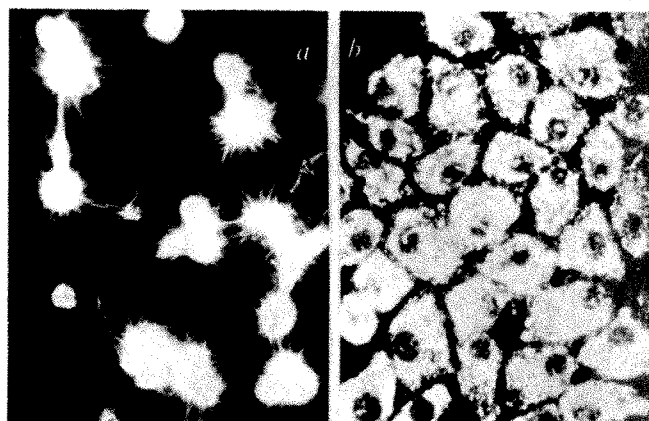


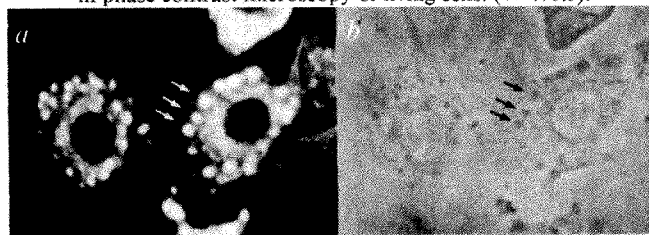
Fig. 1 Immunofluorescent staining of chondrocytes from 16-day embryonic chick sterna with antibodies against type II collagen after 24 h in culture. Chondrocytes were released from sterna by treatment with 0.25% trypsin and 0.1% crude collagenase for 90 min at 37 °C, washed with F12 medium plus 10% foetal calf serum, and plated on Falcon plastic dishes No. 3001 at cell densities of 10^4 cells per cm^2 in Ham's F12 medium and 10% foetal calf serum^{1–3}. After 24 h most cells had attached to the surface, but none were flattened; they showed strong membrane-bound fluorescence and numerous filopodia. For immunofluorescent staining, cells were washed with saline and fixed with 70% ethanol followed by 98% ethanol-ether (1:1, v/v). The air dried cells were reacted with monospecific rabbit antibodies to chick type II collagen (0.12 mg ml^{-1}) for 30 min at room temperature, washed and counterstained with fluorescein conjugated goat anti-rabbit γ globulin as described earlier⁹. Preparation and specificity of the antibodies was described previously⁹. Cells did not stain with antibodies to type I collagen at this time in culture. $\times 260$. **b**, Immunofluorescent staining (see Fig. 1) of a chondrocyte colony after 48 hours in culture. Cells have flattened and retracted their filopodia. Fluorescence with antibodies to type II collagen is located mostly intracellularly as indicated by the weak staining of the membrane above the nuclear area. $\times 260$.

large vacuoles in some cells (Fig. 2). These vacuoles appeared dark in the phase contrast microscope after fixation and antibody staining (Fig. 2b) but were not visible in living cells. It cannot be ruled out that they were an artefact of the fixation and drying procedure, but we assume that they are secretory granules filled with procollagen¹².

After 2 d in culture the first cells started to produce type I collagen. Biochemical determinations have shown that after 2 weeks about 50% of the newly synthesised collagen was type I collagen^{1–3}. Double staining with antibodies to type I and type II collagen revealed that most cells synthesised either type I or type II collagen (Fig. 3). Only in about 1 out 100 cells was fluorescence observed with both antibodies. There was no significant difference in the morphologic appearance of type I and type II collagen synthesising cells. We observed cells of nearly identical shape in close proximity, which may have originated from the same mother cell, one synthesising type I and the other type II collagen (Fig. 3, arrows).

Type I as well as type II collagen synthesising binucleated cells were observed in increasing amount with prolonged

Fig. 2 Immunofluorescence (a) and phase contrast micrograph (b) of the same cells of a 12-day chondrocyte culture after fixation and staining with antibodies to type II collagen. Fluorescence seems to focus in large intracellular vacuoles (arrows) which may be secretory granules containing type II procollagen. The phase contrast is low because the cells were fixed and air dried. This kind of vacuole was not seen in phase contrast microscopy of living cells. ($\times 478.5$).



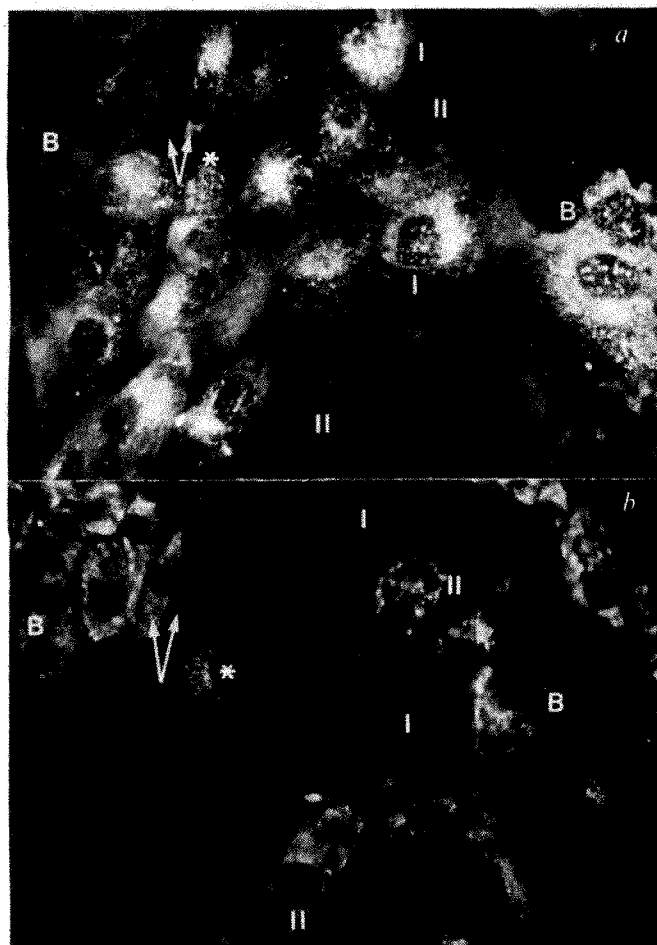


Fig. 3 Immunofluorescent double staining of a chondrocyte culture after 14 d in culture with rabbit antibodies to type I collagen followed by rhodamin conjugated goat anti-rabbit γ -globulin, and guinea pig antibodies to type II collagen followed by fluorescein conjugated rabbit anti-guinea pig γ -globulin⁹. In (a) the fixed culture was photographed with a red filter Rfl 580 (Zeiss), revealing only type I collagen-bound rhodamine fluorescence, and (b) shows type II collagen-bound fluoresceine reaction of the same cell group, photographed through a KP490 filter (Zeiss)⁹. Most cells synthesise either type I (I) or type II (II) collagen except for the cell labelled by an asterisk (*) which shows fluorescence with both types of antibodies. Type I and type II collagen synthesising cells are often located in close proximity and of nearly identical cell shape (arrows). Binucleated cells (B) may produce type I or type II collagen. $\times 360$.

time in culture (B in Fig. 3), which may have been created by incomplete mitosis or cell fusion.

This experiment demonstrates, first, that no conclusions can be drawn from the morphological appearance of a cultured chondrocyte about the type of collagen the cell is producing. Second, because only a few cells were observed which synthesised type I and type II collagen simultaneously, it is evident that the cells do not change gradually from type II to type I collagen synthesis, but rather abruptly. Since the overall transition from type II to type I collagen synthesis in a culture is a gradual process extending through several weeks¹⁻⁴, individual cells must 'switch' at different times in culture. This is consistent with the observation of an increasing number of type I collagen producing cells during the cell culture period, associated with a decreasing number of type II collagen producing cells.

The reason for individual differences in the stability of the chondrocyte phenotype within the cell population obtained after dissociation of embryonic sterna is still unclear. It is possible that young chondroblasts derived from regions underneath the perichondrium had to undergo more doublings to dedifferentiate than did older chondrocytes from the centre of the sternum which were closer to hypertrophy than subperichondral cells. We have also

observed that in general cells located at the periphery of cartilage making colonies³ or cells in between those had changed to type I collagen synthesis earlier than cells from the centre of the colony. This indicates that the stability of the chondrocyte phenotype is also dependent on cell-cell or cell-matrix interactions.

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Effect of prostaglandin endoperoxides and metabolites on bone resorption *in vitro*

PROSTAGLANDINS are potent bone resorbers¹ which have been implicated as mediators of bone loss and hypercalcaemia in disease²⁻⁶, but the specific products in the pathway of prostaglandin synthesis and degradation which are responsible have not yet been identified *in vivo*. Although PGE₂ is the most potent stimulator of bone resorption among the prostaglandins thus far tested *in vitro*⁷, in animal tumour models, the increase in immunoreactive PGE₂ concentrations does not correlate well with the development of hypercalcaemia². We therefore tested the early products of arachidonic acid metabolism via the cyclo-oxygenase pathway, the prostaglandin endoperoxides (PGG₂ and PGH₂) and some of the 13,14-dihydro (H-PGE₂ and H₂-PGE₁) and 15-keto-13,14-dihydro metabolites of prostaglandins (15K-H₂-PGE₂ and 15K-H-PGF_{2α}) for their effects on the release of previously incorporated ⁴⁵Ca from cultured foetal rat long bones. The endoperoxides were found to cause a rapid transient increase in the release of previously incorporated ⁴⁵Ca from bone, but did not stimulate prolonged resorption. H₂-PGE₂ and H₂-PGE₁ were almost as potent as the parent compounds. 15K-H₂-PGE₂ and 15K-H-PGF_{2α} were much less potent, but did stimulate resorption at 10⁻⁶ M.

The diseases in which increased prostaglandin production has been implicated as the mediator of pathologic bone loss include malignancy^{2,3}, rheumatoid arthritis⁴, and periodontal disease^{5,6}. Bone itself can produce prostaglandin when cultured with antibodies to cell surface components and complement⁸. Moreover, PGE₂ can inhibit bone collagen synthesis as well as stimulate bone resorption⁹.

There is clear evidence for an increased metabolism of arachidonic acid via the cyclo-oxygenase pathway in the hypercalcaemia of malignancy, as markedly increased levels of PGE₂ metabolites have been found in the blood of hypercalcaemic mice with the HSDM₁ fibrosarcoma¹⁰ and hypercalcaemic rabbits bearing the VX₂ tumour¹¹ and in the urine of patients with the hypercalcaemia of cancer². It has been more difficult, however, to prove that PGE₂ itself is the circulating material responsible for hypercalcaemia. In animal tumour models a modest increase in immunoreactive PGE₂ is seen only after hypercalcaemia has developed², and in patients

with the hypercalcaemia of cancer the evidence for increased circulating immunoreactive PGE_2 is conflicting^{12,13}. Low levels of circulating PGE_2 in the presence of its increased synthesis are most likely the result of its rapid metabolism by the lung and other tissues so that PGE_2 itself may not accumulate in the circulation¹⁴. This raises the possibility that either metabolites of PGE_2 or other products of arachidonate metabolism via the cyclo-oxygenase pathway such as thromboxane or prostacyclin¹⁵ might stimulate osteoclastic activity and produce hypercalcaemia.

Accordingly, we have evaluated the bone resorption produced by PGG_2 and PGH_2 , the labile cyclic endoperoxides from which all prostaglandins and thromboxanes are formed¹⁶. We have also examined the major metabolite of PGE_2 that persists in the circulation, 15-keto-13,14-dihydro- PGE_2 ($15\text{K-H}_2\text{-PGE}_2$), the major product of metabolism by the lung, as well as 13,14-dihydro- PGE_2 ($\text{H}_2\text{-PGE}_2$) which is produced by reduction of the 15-keto group of $15\text{K-H}_2\text{-PGE}_2$ in organs such as the liver¹⁷. Finally, we compared some analogous compounds, PGE_1 , $\text{H}_2\text{-PGE}_1$ and $15\text{K-H}_2\text{-PGF}_{2\alpha}$.

The endoperoxides, PGG_2 and PGH_2 , were prepared according to the method of Hamberg *et al.*¹⁸; following isolation by silicic acid chromatography their purity and structures were confirmed by thin layer chromatography and mass spectrometry of their respective reduction and isomerisation products¹⁹. $\text{H}_2\text{-PGE}_2$, $\text{H}_2\text{-PGE}_1$, $15\text{K-H}_2\text{-PGE}_2$ and $15\text{K-H}_2\text{-PGF}_{2\alpha}$ were prepared by incubation of ^3H -labelled and unlabelled precursors with homogenates of guinea pig liver by the method of Hamberg and Israelsson¹⁷. The products were resolved initially by reversed phase partition chromatography with solvent system C-50 (ref. 20) and further purified with high performance liquid chromatography²¹. The structures were confirmed by mass spectrometry. PGE_2 and its metabolites were quantified on the basis of radioactivity after standardisation by gas chromatography.

The effects of prostaglandin endoperoxide and metabolites on bone resorption were assessed by measuring the release of previously incorporated ^{45}Ca from foetal long bones cultured in a chemically defined medium with 4 mg ml⁻¹ bovine serum albumin as described previously^{7,22}. The bones were precultured for 18–24 h to remove exchangeable ^{45}Ca . The endoperoxides were stored at -80°C in acetone and were injected into the culture medium in 1 μl of acetone per 500 μl of medium. Controls received acetone alone. The metabolites were added in alcohol (less than 0.1% final concentration). Controls received alcohol alone. ^{45}Ca release was measured in medium and bone and the per cent of total bone ^{45}Ca released during each culture period calculated.

The prostaglandin endoperoxides, PGG_2 and PGH_2 , did not produce consistent stimulation of bone resorption in 48-h cultures when injected at 3–12 h intervals. Their effect was always less than that of equivalent amounts of PGE_2 administered in an identical manner. Because of their short half life, the effects of more frequent injections were examined. Bone cultures were injected hourly with endoperoxides or PGE_2 for 5 h and calcium release measured at 6 h. The cultures were then transferred to fresh medium to see if further resorptive responses occurred. Using this schedule, PGG_2 at 10^{-6} and 10^{-8} M and PGH_2 at 10^{-6} and 10^{-7} M increased the release of ^{45}Ca from foetal bones at 6 h (Table 1). The effect of PGH_2 at 10^{-8} M was tested in four additional experiments; in two of these ^{45}Ca release was increased significantly by 20–25%. Hence 10^{-8} M PGH_2 is apparently at or near the threshold concentration for early ^{45}Ca release. PGE_2 at 10^{-6} to 10^{-8} M did not produce a significant 6-h increase in ^{45}Ca release in any experiment. After 6 h of treatment with PGE_2 , however, there was always a significant resorptive response during the subsequent 42 h of culture. The 6–48-h response after endoperoxide treatment was quite variable and usually not statistically significant. A prolonged response to brief exposure has been observed with other stimulators of bone resorption²³ and has been termed induction. Because the rapid effects of

Table 1 Effect of PGG_2 , PGH_2 and PGE_2 on release of previously incorporated ^{45}Ca from cultured foetal rat long bones

Treatment	% of total bone ^{45}Ca release 0–6 h	0–48 h
Experiment 1		
Control	8.2±0.4	21.7±0.6
PGG_2 10^{-6} M × 5	10.7±0.5*	27.5±3.2
10^{-8} M × 5	10.4±0.7*	24.4±1.5
PGH_2 10^{-6} M × 5	12.2±0.7*	30.7±4.2
10^{-8} M × 5	9.2±0.3	20.6±0.5
PGE_2 10^{-6} M × 5	8.4±0.3	30.2±2.4*
10^{-8} M × 5	8.5±0.5	28.0±1.8*
Experiment 2		
Control	8.5±0.4	27.8±2.7
PGH_2 10^{-7} M × 5	15.8±1.2*	29.2±1.9
PGE_2 10^{-6} M × 5	9.2±0.2	45.3±1.8*

Values are means ± s.e. for four to twelve bone cultures treated with 5-hourly injections of prostaglandin in acetone beginning at 0 time; controls received acetone alone. Cultures were transferred to fresh medium without prostaglandins or acetone at 6 h.

*Significantly different from controls, $P < 0.01$.

endoperoxides were small in terms of total bone calcium, it was not possible to determine whether there was stimulation of matrix resorption as well as calcium release at 6 h. The initial effect could have been due to an increase in cell permeability to calcium²⁴. High calcium concentrations in the medium have previously been shown to inhibit induction by parathyroid hormone²⁰. It is possible that the calcium released by endoperoxides could decrease the subsequent resorptive response even if a substantial amount of PGE_2 were produced. It is also possible that the endoperoxides produced tissue damage which impaired resorptive response. We have, however, observed that endoperoxide-treated bone can respond to subsequent addition of both PGE_2 and parathyroid hormone (data not shown).

The metabolites of PGE_2 , PGE_1 and $\text{PGF}_{2\alpha}$ which may have a longer half life in the circulation than the parent compounds were tested only in prolonged continuous cultures. Both $\text{H}_2\text{-PGE}_2$ and $\text{H}_2\text{-PGE}_1$ were nearly as potent as the parent compounds (Fig. 1) and as previously reported⁸ PGE_1 was somewhat less potent than PGE_2 . In contrast, $15\text{K-H}_2\text{-PGE}_2$ and $15\text{K-H}_2\text{-PGF}_{2\alpha}$ were much less effective. Some stimulation of resorption was observed at 10^{-5} M (Table 2). In another experiment 89% of total ^{45}Ca released after 5 d treatment with $15\text{K-H}_2\text{-PGE}_2$ at 2×10^{-5} M, but at 2×10^{-4} M, the highest concentration tested, $15\text{K-H}_2\text{-PGE}_2$ actually inhibited ^{45}Ca

Fig. 1 Effect of prostaglandins E_2 (●) and E_1 (■) and their 13,14-dihydro metabolites, $\text{H}_2\text{-PGE}_2$ (○) and $\text{H}_2\text{-PGE}_1$ (□), on release of previously incorporated ^{45}Ca from cultured foetal rat long bones. Data from eight experiments were pooled and the points are means for 6–22 cultures incubated for 5 d with medium changed at 2 d. Standard errors were 5 to 15% of the means. The differences from control at 10^{-7} and 10^{-6} M were significant at $P < 0.01$, at 10^{-8} M at $P < 0.05$. Broken line represents control levels. a, Release at 2 d; b, release at 5 d.

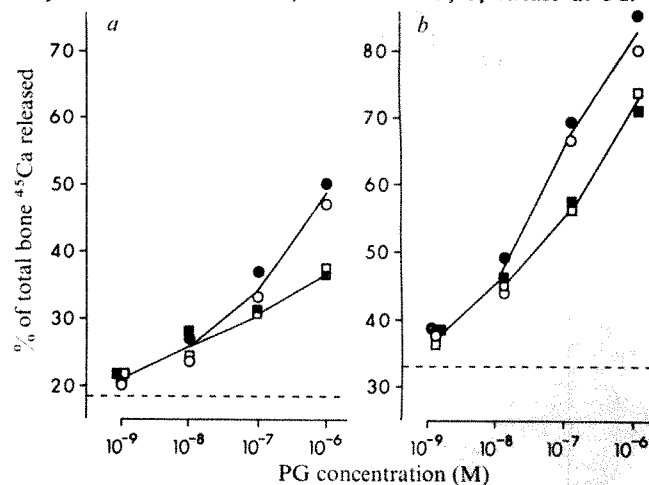


Table 2 Effect of 15K-H₂-PGE₂ and 15K-H₂-PGF_{2α} on release of previously incorporated ⁴⁵Ca from cultured foetal rat long bones

Treatment	% of total bone ⁴⁵ Ca released
Control	40.3 ± 4.1
15K-H ₂ -PGE ₂ 10 ⁻⁷ M	43.8 ± 5.0
10 ⁻⁶ M	48.0 ± 6.4
10 ⁻⁵ M	54.1 ± 2.9*
15K-H ₂ -PGF _{2α} 10 ⁻⁷ M	38.0 ± 4.3
10 ⁻⁶ M	38.2 ± 3.7
10 ⁻⁵ M	55.5 ± 6.4*

Values are mean ± s.e.m. for four to eight bones cultured for 5 d. Medium was changed at 2 d.

*Significantly different from controls, $P < 0.05$.

release to 25% compared with a control of 41%. The inhibition may be a toxic effect and has been seen with other prostaglandins at high concentrations⁸. At their maximally effective doses, all the agents tested produced similar morphological changes with almost complete resorption of mineral and matrix at 5 d. To exclude any possible contribution of different binding to albumin to the relative potencies of these compounds, a separate experiment was performed in which medium without albumin was used, and PGE₂ stimulated bone resorption significantly at 10⁻⁸ M while 15K-H₂-PGE₂ was again ineffective at 10⁻⁶ M. These results indicate that the 13,14-dihydro derivatives are nearly as potent stimulators of bone resorption as PGE₂ and PGE₁, whereas 15K-H₂-PGE₂ is considerably less potent. Atkins and Martin²⁵ found a similar relation between metabolites and parent compounds for stimulation of cyclic AMP production by osteogenic sarcoma cells. It will be of interest to determine whether any of these compounds can accumulate sufficiently in the circulation to contribute to the resorption of bone in the hypercalcaemia of cancer.

The prostaglandin endoperoxides, PGG₂ and PGH₂, cause a transient stimulation of calcium release that is quite different from the prolonged resorption resulting from PGE₂. These findings raise the possibility that bone tissue enzymically converts PGG₂ and PGH₂ to a labile product such as thromboxane A₂ or prostacyclin (PGX). Further studies will be required to delineate which of the products of arachidonate metabolism via the cyclooxygenase pathway account for the resorption of bone in neoplasia and inflammation.

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Increased cyclic GMP levels associated with contraction in muscle fibres of the giant barnacle

CYCLIC GMP has been implicated as an intracellular second messenger in a variety of systems. In particular, a number of hormones and neurotransmitters have been shown to raise cellular cyclic GMP levels *in vitro*^{1–9}. In many of these systems, however, Ca²⁺ entry seems to be responsible both for the physiological effect of the hormone and for the hormone-stimulated increase in cyclic GMP (refs 4–9). We have investigated the relationship between calcium and cyclic GMP levels in muscles of the giant barnacle *Balanus nubilus*. This preparation has a number of advantages. Within fairly narrow limits intracellular free calcium levels in the resting muscle are known¹⁰. The depolarisation-induced increase in free calcium, and the resulting activation of contraction, have also been extensively documented^{10,11}. In addition, the depolarisation-induced changes in intracellular free calcium can be easily manipulated because the extracellular fluid is the apparent source of the calcium which couples excitation to contraction¹². We report here that either KCl depolarisation or nerve stimulation increases the cyclic GMP content of barnacle muscle. This increase seems to be associated with Ca²⁺ entry rather than with a neurotransmitter–receptor interaction *per se*. This is the first report of an increase in cyclic GMP associated with contractile activity in cross-striated muscle.

The three pairs of depressor muscles were used in all the experiments described below. The dissection of these muscles and their nervous supply has been described previously¹³. The cyclic GMP content of resting barnacle muscle, measured according to the procedure described in Fig. 1, was 75 ± 8 fmol per mg protein (mean ± s.e.m. for 34 individual resting muscles from a total of 18 barnacles).

Depolarisation of the muscles by elevating the KCl concentration of the barnacle saline for 2 min produced an increase both in isometric tension and in cellular cyclic GMP (Fig. 1). The isometric tension increased rapidly and reached a near-maximal value within 15 s. The cyclic GMP content was measurably elevated within 30 s and continued to increase until 2 min, at which time it was nearly three times control. Beyond this time, the cyclic GMP level fell towards that of resting muscle, even when the exposure to KCl was maintained. The cyclic GMP content declined toward the basal level despite the fact that the muscle was able to maintain a constant isometric tension for as long as the KCl stimulus was present (longest time tested = 5 min, data not shown). These results indicate that the cyclic GMP level does not directly control the contractile state. (Although we have not established the reason for the decline in cyclic GMP, it may be physiologically important; for instance, Ca²⁺ entry or some other event associated with muscle contraction might cause a rapid increase in the synthesis, and a slower increase in the degradation, of the nucleotide.) If, after 2 min of KCl depolarisation, the muscles were returned to normal barnacle saline, isometric tension decreased to the pre-stimulus level and the fall in cyclic

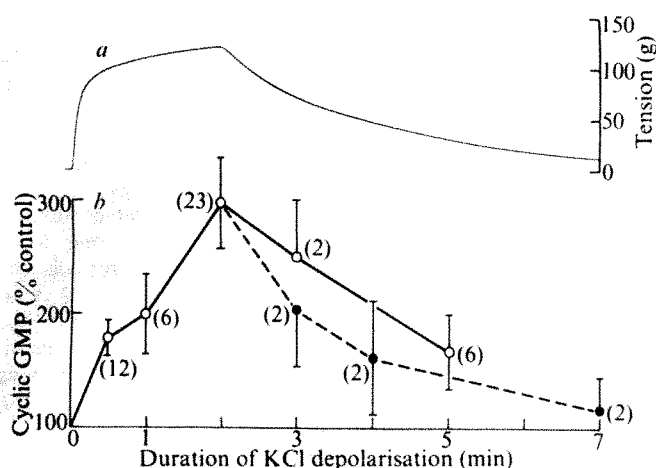


Fig. 1 Effect of KCl depolarisation on isometric tension (a) and cyclic GMP content (b) of giant barnacle muscle. The tension trace shows the effect of a 2-min exposure of the muscle to 100 mM KCl-barnacle saline followed by a return to control barnacle saline. The control barnacle saline (modified from ref. 13) had composition (mM): Na^+ , 476; K^+ , 8; Ca^{2+} , 20; Mg^{2+} , 12; Cl^- , 548; MOPS (3-(*N*-morpholino)propanesulphonic acid), 10; pH adjusted to 7.4 with NaOH. The 100 mM KCl-barnacle saline was made by equimolar substitution of K^+ for Na^+ in the control saline. The cyclic GMP data were calculated as follows: from each barnacle 1–5 muscles were used as test muscles and 1–3 muscles as control muscles. The cyclic GMP content of an individual test muscle was expressed as $100 \times (\text{pmol cyclic GMP per mg protein of the test muscle} \div \text{mean pmol cyclic GMP per mg protein of control muscle from the same barnacle})$. Thus, each barnacle yielded 1–5 experimental values. ○, Mean percentage ($\pm$ s.e.m.) cyclic GMP content, relative to control as described, for test muscles incubated for the indicated duration in 100 mM KCl-barnacle saline; ●, mean percentage cyclic GMP content, relative to control, of muscles returned to normal barnacle saline after 2 min in 100 mM KCl-barnacle saline. In all cases, control muscles were incubated in normal barnacle saline for a time equivalent in duration to the incubation of experimental muscles. All muscles were dissected and pre-incubated (1–3 h at 4 °C) in control barnacle saline. Experiments were carried out at room temperature (19–21 °C). After experimental incubations, cyclic nucleotides were extracted by rapidly homogenising the muscles with an Ultraturrax in 7–10 ml ice-cold 7% TCA. Alternatively, the muscles were first frozen in liquid nitrogen and then homogenised in TCA. The cyclic GMP level of resting muscle did not depend on which of these two treatments was used. The TCA-precipitated proteins were pelleted by centrifugation; after removing the supernatant the protein content of the pellets was measured by the method of Lowry *et al.*²². Aliquots (3 ml) of the supernatant were extracted five times with 10 ml water-saturated ether, lyophilised and reconstituted with 0.5 ml 50 mM sodium acetate, pH 6.2. Cyclic GMP was determined by the method of Steiner *et al.*²³ and cyclic AMP by the method of Brown *et al.*²⁴. The recovery of exogenous cyclic GMP and cyclic AMP, added to whole muscle homogenates, was 85% and 90%, respectively. Treatment of the sodium acetate-reconstituted samples with beef heart phosphodiesterase (Boehringer) reduced their cyclic nucleotide content essentially to zero.

GMP seemed to be slightly more rapid than in the continued presence of high KCl although the difference was probably not statistically significant (Fig. 1).

KCl depolarisation seemed to have relatively little effect on the cyclic AMP content of barnacle muscle, but these data are less complete. The basal cyclic AMP level was 3.57 ± 0.41 pmol per mg protein (mean $\pm$ s.e.m.; 12 muscles from 7 barnacles). Depolarisations ranging from 30 s to 2 min caused an increase of $19 \pm 6\%$ above control (14 muscles from 7 barnacles).

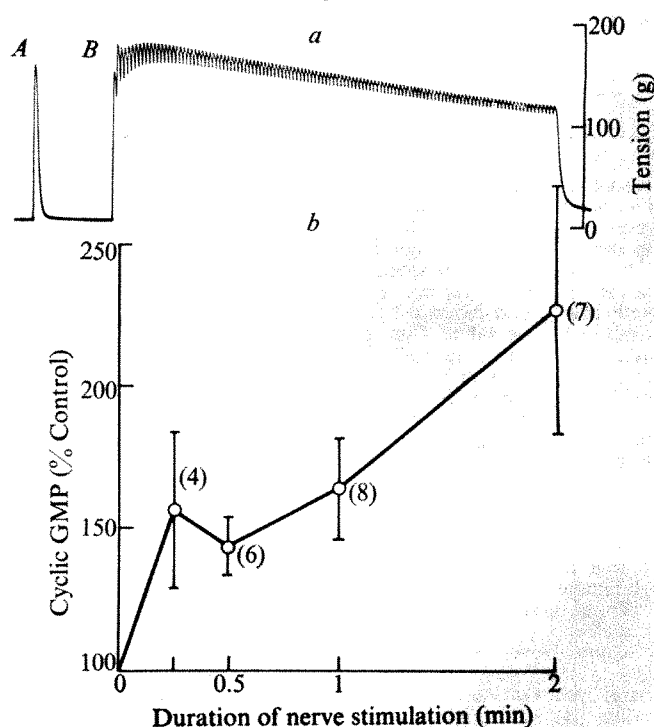
The effect of nerve-evoked contraction on the intracellular level of cyclic GMP is shown in Fig. 2. Repetitive nerve stimulation caused a maintained increase in tension, and a time-dependent increase in muscle cyclic GMP, during the interval studied.

To determine whether, as in many other systems⁴⁻⁹, the stimulated increases in barnacle muscle cyclic GMP level are

dependent on extracellular Ca^{2+} , muscles were soaked for 2–3 h in Ca^{2+} -free barnacle saline. After such treatment, depolarisation with Ca^{2+} -free 100 mM KCl-barnacle saline caused no visible contraction and no increase in cyclic GMP (Table 1, B1). The absence of an increase in cyclic GMP was not a result of deterioration of the muscle. Exposing muscles which had been preincubated in Ca^{2+} -free barnacle saline to Ca^{2+} -containing 100 mM KCl-barnacle saline caused a strong contraction and an increase in cyclic GMP comparable to that for muscles which had been preincubated in normal barnacle saline (compare B2 with A2 in Table 1).

Based on studies of other crustaceans¹⁴, glutamate is probably the excitatory neuromuscular transmitter in the barnacle. As support for this contention we found that bath applied glutamate (0.1–1.0 mM) caused barnacle muscle to contract; but this contraction was weak and variable, with a duration of usually less than 10 s. In such conditions (0.5 mM glutamate for 2 min), there was no alteration in muscle cyclic GMP ($101 \pm 18\%$, relative to control, for six muscles). The bath application of higher (10 mM) concentrations of glutamate caused a vigorous, brief (<1 s) contraction. The brevity of this contraction is probably a consequence of the rapid desensitisation of the muscle glutamate receptors. This desensitisation to applied glutamate was accompanied by a rapid reduction in the visible contraction induced by nerve stimulation, further supporting the hypothesis that glutamate is the excitatory neurotransmitter. Complete abolition of the response to nerve stimulation required an exposure to 10 mM glutamate

Fig. 2 Effect of nerve stimulation of varying duration on isometric tension (a) and on cyclic GMP content (b) of giant barnacle muscle. The time scale applies to parts A and B of the tension record as well as to the cyclic GMP data. a, A, Isometric tension response to a single stimulus train applied to the motor nerve. An individual train consisted of 10 pulses delivered at 100 Hz; each pulse was supramaximal in amplitude and 1 ms in duration. a, B, Isometric tension response when such a stimulus train was applied repetitively (once per second for 2 min). b, Each point shows the mean percentage ($\pm$ s.e.m.) of cyclic GMP in stimulated muscles compared with control muscles (calculated as described in Fig. 1). Stimulus conditions were identical to those for a, B. After stimulating the nerve for the indicated periods of time, the muscles (both stimulated and control) were homogenised and further processed as described in Fig. 1.



for 1 h. (This slow time course does not seem unreasonable since many of the individual fibres of the muscle are not directly exposed to the bathing solution and since many of the neuromuscular junctions of individual fibres lie at the bottom of deep, narrow clefts¹⁵.) After neuromuscular transmission was blocked with 10 mM glutamate, stimulation of the motor nerve no longer caused an increase in muscle cyclic GMP level (Table 1, C1). This observation suggests that the nerve-induced (Table 1, A1) increase in cyclic GMP requires the activation of glutamate receptors by the neurotransmitter. KCl depolarisation, however, was still able to increase the cyclic GMP level in glutamate-desensitised muscles (Table 1, C2). This finding indicates that the KCl-induced increase in cyclic GMP is not a result of depolarisation-dependent release of glutamate from the nerve terminals. The simplest hypothesis that could account for the increase in cellular cyclic GMP produced by nerve stimulation and KCl depolarisation, as well as for the fact that the KCl-induced increase required Ca^{2+} , is that both types of contractile stimuli increase cyclic GMP by causing Ca^{2+} influx across the muscle membrane.

In the barnacle, as well as in a number of other systems, receptor activation by the appropriate hormone or neurotransmitter is associated with increased cyclic GMP and a physiological response. In some systems, cyclic GMP seems to serve as a causal link between receptor activation and the physiological response. For example, muscarinic cholinergic agonists increase cyclic GMP levels in the heart¹ and in sympathetic ganglia⁵, and exogenous derivatives of cyclic GMP are able to mimic some of the physiological effects of muscarinic agonists in these preparations^{16,17}. In certain other systems in which hormonal elevation of cyclic GMP has been observed, Ca^{2+} seems, however, to link stimulus to response^{3,4,8-8}. It seems likely that the barnacle muscle is one of these latter systems and that calcium rather than cyclic GMP is the agent which directly activates the contractile machinery.

Table 1 Effect of nerve stimulation and of KCl depolarisation on cyclic GMP in giant barnacle muscle in various conditions

Pre-incubation	Stimulation	% Control
A, Control saline	1. Nerve stimulation	227 ± 44 (7)
	2. 100 mM KCl-saline with Ca^{2+}	298 ± 39 (23)
B, Ca^{2+} -free saline	1. 100 mM KCl without Ca^{2+}	99 ± 5 (8)
	2. 100 mM KCl with Ca^{2+}	370 ± 49 (11)
C, Glutamate saline	1. Nerve stimulation	119 ± 13 (5)
	2. 100 mM KCl-glutamate-saline	267 ± 60 (18)

A, Muscles were dissected, and pre-incubated for 1–3 h, in control barnacle saline, as described in Fig. 1. Control muscles were then incubated for an additional 2 min in control barnacle saline; stimulated muscles were incubated for 2 min in 100 mM KCl-barnacle saline or were stimulated through their motor nerve as described in Fig. 2.

B, Muscles were dissected and washed with Ca^{2+} -free barnacle saline (equimolar replacement of the Ca^{2+} in the control saline with Mg^{2+}) and incubated for 2–3 h at 4 °C, with two changes of fresh Ca^{2+} -free saline. Control muscles were then incubated for an additional 2 min in Ca^{2+} -free saline; stimulated muscles were incubated for 2 min in Ca^{2+} -free 100 mM KCl-saline or for 2 min in Ca^{2+} containing 100 mM KCl-saline.

C, All muscles were pre-incubated for 1–3 h at 4 °C in control barnacle saline, and then incubated for an additional hour at 20 °C in barnacle saline plus 10 mM monosodium glutamate ('glutamate saline'). Control muscles were then incubated for an additional 2 min in glutamate saline. Stimulated muscles were either maintained for 2 min in glutamate saline, during which time the motor nerve was stimulated as described in Fig. 2, or else were incubated for 2 min in 100 mM KCl-saline containing 10 mM monosodium glutamate.

Data: mean value ± s.e.m. (number of individual experimental muscles).

Control levels of cyclic GMP were: A, 75 ± 8 ($n = 34$); B, 43 ± 4 ($n = 16$); C, 62 ± 6 ($n = 18$) fmol per mg protein. Note that the basal cyclic GMP level in muscles pre-incubated in Ca^{2+} -free barnacle saline (B) was only about half that of muscles pre-incubated in normal saline (A).

Since Ca^{2+} entry seems to be responsible both for the activation of contraction in the barnacle, and for the increase in cyclic GMP, the problem remains as to the function of the cyclic GMP increase associated with contractile activity in the muscle fibres of the barnacle. One possibility^{18,19} is that cyclic GMP is involved in regulating Ca^{2+} metabolism. Ong and Steiner²⁰ have suggested that cyclic GMP may have a role in the regulation of myosin function. Another possibility worth considering is that cyclic GMP has a role in activity-dependent control of the expression of the muscle genome. In mammalian skeletal muscle, for example, muscle activity can prevent the appearance of denervation-induced extrajunctional acetylcholine receptors²¹. In preliminary experiments, we have found that contractile activity in vertebrate skeletal muscle is associated with an increase in cyclic GMP level.

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Development of cholinergic characteristics in adrenergic neurones is age dependent

OBSERVATIONS from several laboratories indicate that neurones from adrenergic ganglia cultured from rats during the perinatal period may, in certain culture conditions, develop several clearly cholinergic characteristics. These cultured neurones may form cholinergic synapses among themselves as well as on cocultured striated muscle^{1–3}, and the cell population developing cholinergic mechanisms derives from an initially adrenergic neuronal population as a gradual shift in synaptic vesicle cytochemistry occurs together with increasing choline acetyltransferase (ChAc) activity and an increasing number of cholinergic synaptic interactions between neurones^{4,5}. In certain conditions these cultured neurones may release both noradrenaline and acetylcholine⁶. We address here the question of whether this unexpected ability of the adrenergic neurone to develop cholinergic mechanisms in culture derives from the neurone's response to some novel aspect of the tissue culture system

or is instead the *in vitro* expression of a developmental period during which the automatic neurone *in vivo* is in a labile state of differentiation. Our results favour the latter explanation.

These experiments were made possible by two developments. First, small explants of superior cervical ganglia (SCG) were successfully cultured from increasingly older

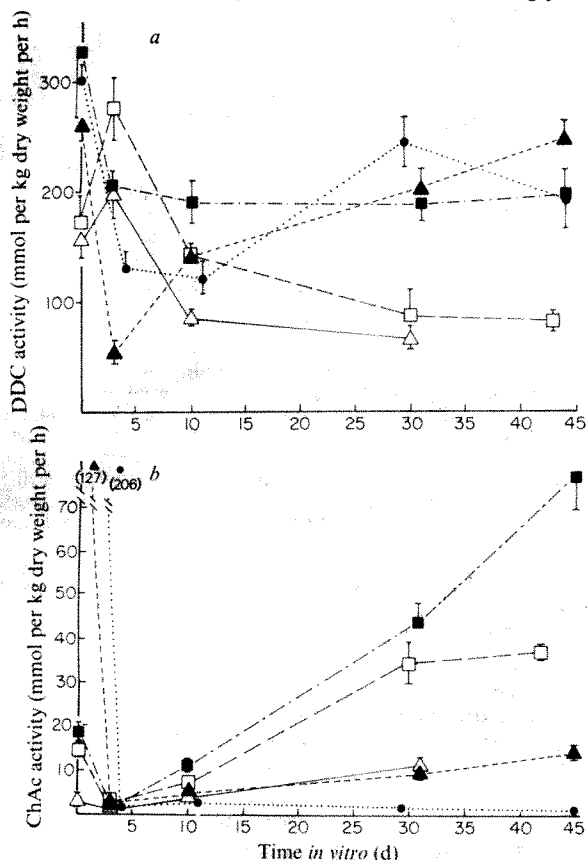


Fig. 1 *a*, Variation of DOPA-decarboxylase (DDC) activity in SCG explants with time in culture. Ganglia were obtained from 15½-d (△) and 20-d (□) day embryonic and 2-d (■), 5-d (▲), 22-d (●) (not shown), and 200 (●) day postnatal rats. Explants of approximately equal size were obtained by culturing the entire 15½-d embryonic ganglion and by dividing the increasingly larger, more mature ganglia into pieces of similar size. Explants were placed on to 24-mm Aclar dishes and fed three times weekly with medium containing 65% Eagle's minimum essential medium with glutamine added, 25% human placental serum (HPS) 10% chick embryo extract (EE), 3% 1.1 M glucose, and 25 units ml⁻¹ nerve growth factor. These levels of HPS and EE have been shown to foster the development of cholinergic properties in cultures of SCG neurones⁷. At the times indicated, cultures were rinsed with Leibovitz medium and, after carefully blotting excess medium, were frozen and stored at -80 °C. Cultures were freeze-dried under vacuum at -40 °C (ref. 18) for 3 d. Explants were dissected free from supporting cell and neurite outgrowth and from the collagen, weighed using a quartz-fibre balance¹⁸, and homogenised in 36 µl of 50 mM sodium phosphate buffer (pH 7.6) containing 0.1% bovine serum albumin and 0.1% Triton X-100. Intact ganglia for *in vivo* (or zero time) enzyme activities were treated in the same way except that ganglia from older animals were homogenised in larger volumes in order to keep the weight-volume ratio constant. Homogenates were stored at -80 °C until as said. DDC activity was determined by a modification of the method of McCaman *et al.*¹⁹. Enzyme activity is expressed relative to dry weight of the explant ± s.e.m. There were no significant differences between weights of explants from different age rats after the same time in culture. The DDC activity in explants from postnatal animals after 30 d in culture stabilises at about 70% of the activity in the adult ganglia *in vivo*. Studies of thionin-stained explants are in progress to determine the number of neurones in explants from different age animals after 40 days in culture. *b*, Variation of choline acetyltransferase (ChAc) activity in SCG explants with time in culture. ChAc activity was determined by a modification²⁰ of published methods^{21,22} on the same homogenates described above. Enzyme activity is expressed relative to dry weight of the explant ± s.e.m. Activity at zero time is the *in vivo* activity in the ganglion.

rats, including adult. A similar pattern of ChAc development was seen in SCG from perinatal rats regardless of whether explants or dissociated neurones were studied⁷. Second, we observed that ChAc, the enzyme synthesising acetylcholine and highly localised to cholinergic neurones, is present only in extremely low amounts in all cultures freshly prepared from rat SCG (after degeneration of the pre-ganglionic endings). Thus it was possible to measure ChAc accrual in cultures from adrenergic ganglia prepared at ages between the 15-d embryo and the adult rat. Our results indicate that the ability of SCG explants to develop cholinergic characteristics reaches a maximum at approximately the second postnatal day; beyond the third postnatal week this 'shift' does not occur.

Explants of approximately equal size were prepared from SCG of rats of different ages, cultured in the presence of NGF for 1 month or longer and then assayed for activities of ChAc and of DOPA-decarboxylase (DDC) (details in Fig. 1 legend). The latter enzyme, in the synthetic pathway of catecholamines, is localised to adrenergic neurones in the SCG⁸⁻¹⁰. The cytochemistry of the synaptic vesicle population was studied in cultures from 5-d pups and from adult rats after 1 month in culture by methods previously described⁴.

The pattern of change in DDC activity with time in culture was related to whether the explant was taken from a prenatal or a postnatal rat (Fig. 1*a*). DDC activities in SCG explants from 15½-d and 20-d embryos increased during the first 3 d in culture, then decreased during the following 4 weeks. DDC activities in SCG explants from 2, 15, and 22-d (not shown) pups and from adults first decreased, then levelled or increased during the next 4 weeks, beyond which time there were no significant differences between these explants.

The specific activity of ChAc in SCG explants fell during the first 3-4 d in culture (Fig. 1*b*), probably because of degeneration of presynaptic terminals, then increased during the next 4 weeks in all explants except those taken from the 22-d pup (not shown) and the adult animal. The enzyme activity attained by SCG explants from animals of different ages differed significantly after 1 month in culture; the highest activity was in explants from 2-d pups and the lowest activity was in explants from adults.

The variation with rat age of DDC activity in SCG explants after 1 month in culture differed considerably from the variation in ChAc activity in the same explants (Fig. 2). The accrual of DDC activity by SCG explants increased as explants were taken from progressively older

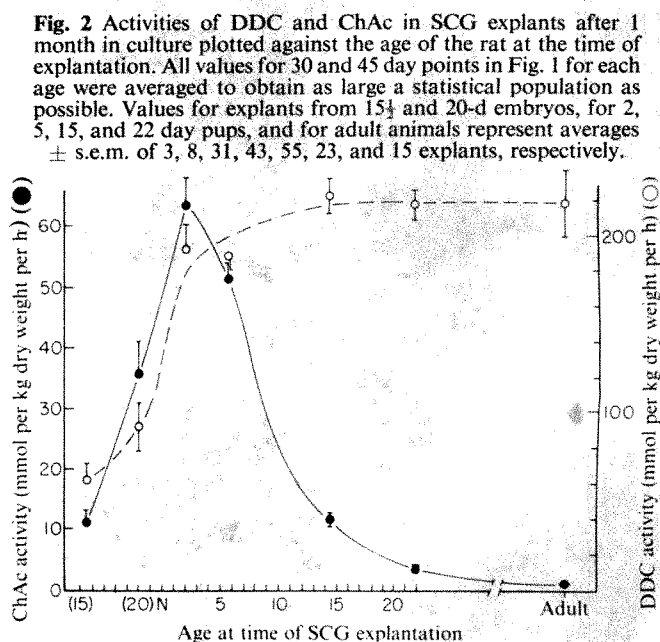


Fig. 2 Activities of DDC and ChAc in SCG explants after 1 month in culture plotted against the age of the rat at the time of explantation. All values for 30 and 45 day points in Fig. 1 for each age were averaged to obtain as large a statistical population as possible. Values for explants from 15½-d and 20-d embryos, for 2, 5, 15, and 22 day pups, and for adult animals represent averages ± s.e.m. of 3, 8, 31, 43, 55, 23, and 15 explants, respectively.

animals from the 15½-d embryo to the 2-d pup; explants from animals aged 2 d or older showed no significant differences in the DDC activity after 30 d or more in culture. The ChAc activity after 30 d in culture was higher as explants were taken from progressively older animals from 15½-d prenatal to 2-d postnatal; but the ability of explants from older animals to develop ChAc activity *in vitro* decreased. The ChAc activity in SCG explants cultured from adult animals did not increase at all, the activity after 45 d being significantly less than the activity remaining 4 d after denervation.

In interpreting these data, we must consider the possibility that our culture system selects certain neurones present in the neonatal animal that are already committed to cholinergic. We have presented biochemical, physiological and cytochemical evidence against this view^{4,5}. In the data presented here, we note no significant differences between the ChAc activities in explants from animals of any age 3 to 5 d after denervation (Fig. 1b, and 5-d and 22-d pups not shown), indicating that no cholinergic cells are present at the time of explantation in neonatal SCG that are not present in the adult. The high level of DDC activity in all explants from postnatal animals (Fig. 2) indicates a good state of neuronal viability, and thus the change with rat age (at explantation) in the accrual of ChAc activity *in vitro* must reflect a difference in the neuronal synthesis of ChAc rather than a difference in survival of selected neuronal populations.

The cytochemistry of vesicles within axonal varicosities was consistent with the ChAc data. Vesicles of varicosities in the outgrowth of SCG explants from 5-d pups were

Fig. 3 Electron micrographs of vesicle-containing varicosities found in cultures of SCG explants taken from *a*, 5-d-old rat pups and *b*, adult rats. The cultures were companions to those used for the biochemical analyses (see Fig. 1 legend) and were fixed after 28–33 d *in vitro*. After an initial rinse in Leibovitz medium (L-15) the cultures were incubated 30 min in 10^{-6} M noradrenalin in L-15 to which ascorbic acid (0.2 mg ml^{-1}) was added. The pH was adjusted to 7.2–7.3 before incubation. A rinse in L-15 was followed by fixation with 3% KMnO_4 in Krebs's Ringer phosphate (pH 7.1, 4°C) and by *en bloc* staining with 1% uranyl acetate in maleic acid buffer (pH 5.2, 4°C). Embedding in Epon-Araldite followed dehydration. Scale bar represents $0.3 \mu\text{m}$.

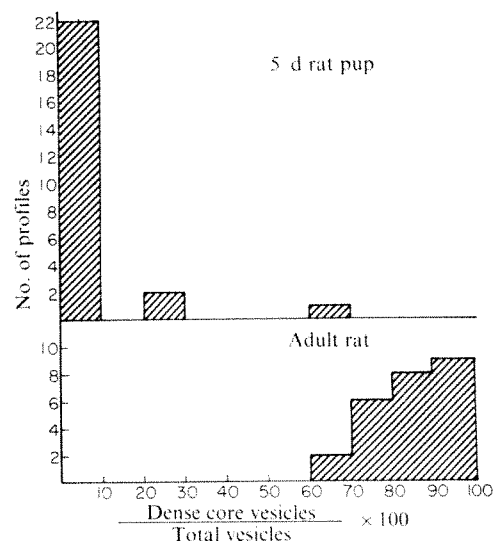
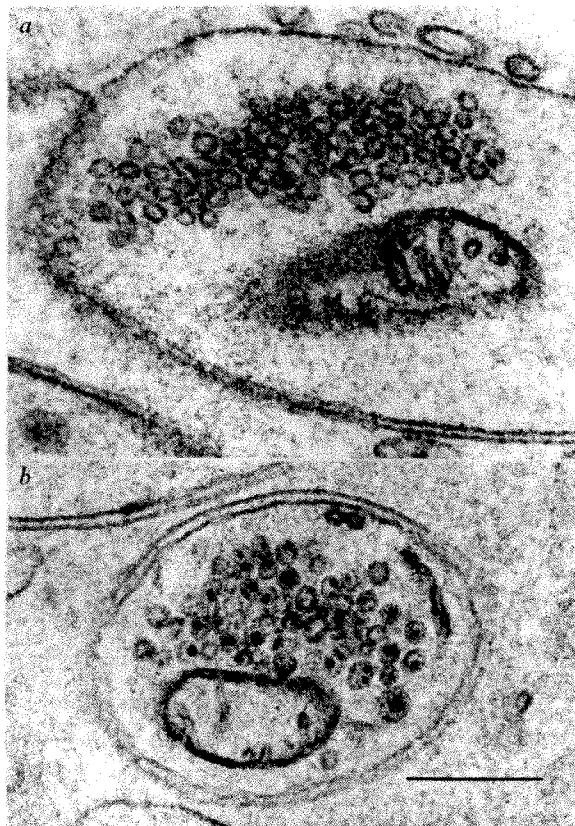


Fig. 4 No. of profiles (ordinate) containing listed percentages of dense-cored vesicles (abscissa) in 28–33-d cultures of SCG from 5-d rat pups and adult rats. Consecutive varicosities, similar to those illustrated in Fig. 3, were photographed and printed at a standard magnification. Vesicles measuring 40–70 nm were counted and classified as dense-cored or clear. Twenty-five profiles from each group are charted. Total vesicles per profile ranged from 9 to 38 and 9 to 54 in the groups taken from 5-d pups and adults respectively.

predominantly clear, whereas the majority of the vesicles of varicosities in explants from adult animals were dense-cored (Fig. 3). This difference is illustrated graphically in Fig. 4. Only 1 of 25 profiles from 5-d pup cultures contained 60–70% dense-cored vesicles and 22 of 25 contained less than 10%. In contrast profiles found in adult rat SCG cultures contained no less than 50% dense-cored vesicles and 17 of 25 contained over 80%.

These data demonstrate that the developmental age of the rat adrenergic neurone determines whether this neurone can adjust its transmitter mechanisms and express in culture two well established characteristics of cholinergic neurones—ChAc activity and clear synaptic vesicles. The dramatic decrease in the ability of SCG explants taken from rats beyond 2 to 5 d postnatal to accrue ChAc activity in culture (Fig. 2) suggests that at this stage in their *in vivo* development sympathetic neurones of the SCG receive important information related to the expression of transmitter mechanisms. A critical event during the differentiation of these neurones apparently begins about 2 d postnatally; the result of this event is a commitment to being adrenergic.

Although these experiments do not rule out the possibility that preganglionic connections influence the expression of cholinergic or adrenergic mechanisms in the sympathetic neurone, available information is more supportive of the view that the target organ, containing adrenergic receptors and perhaps trophic signals (other than nerve growth factor) finally determines the appropriate neurotransmitter these neurones synthesise (ref. 11 for review). The innervation of target organs by the rat SCG is incomplete at birth. Studies involving electron microscopy¹², histochemical fluorescence¹³, norepinephrine uptake¹⁴, and changes in tyrosine hydroxylase activity¹⁵ indicate that sympathetic innervation in iris begins 2–3 d postnatally and that mature innervation in iris is not achieved until 3–4 weeks after birth; in the pineal, mature innervation is not observed until 2–3 weeks after birth^{16,17}. Our tissue culture observations suggest that a signal, most likely from the adrenergic target organ, begins its influence about the second postnatal day in the rat and that this signal ensures the commitment to adrenergic function which characterises most sympathetic neurones *in vivo*.

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Phosphorylation of acetylcholine receptor by endogenous membrane protein kinase in receptor-enriched membranes of *Torpedo californica*

THE molecular basis of synaptic transmission is a fundamental problem in neurobiology. Although neurotransmitters produce striking changes in post-synaptic ion permeability, little is known about the molecular events following binding of neurotransmitter to its receptor. It has been postulated that the effects of neurotransmitters on post-synaptic membranes are mediated by changes in protein phosphorylation^{1,2}. There has been no direct evidence for neurotransmitter-dependent changes in protein phosphorylation in membranes prepared from innervated tissues, however. We investigated this problem using an acetylcholine receptor-enriched membrane fraction prepared from the electric organ of *Torpedo californica* and found that several membrane polypeptides are phosphorylated when this fraction is incubated with γ -³²P-ATP and 100 mM K⁺ (ref. 3). In particular, we demonstrated that a polypeptide of 65,000 molecular weight (MW) is phosphorylated and that phosphorylation of this and other polypeptides is inhibited by cholinergic ligands. A 65,000 MW polypeptide in the same membrane preparation also reacted with antibody prepared against the purified acetylcholine receptor. This suggested that a component of the acetylcholine receptor itself could be phosphorylated by an endogenous membrane protein kinase. Here we present proof that the 65,000 MW component of the acetylcholine receptor is a substrate for an endogenous membrane protein kinase.

Membranes enriched 15-fold in acetylcholine receptors were prepared from the electric organ of *T. californica* as described previously³. Figure 1a shows the polypeptide composition of the acetylcholine receptor-enriched membranes. This fraction contains endogenous protein kinase activity which maximally phosphorylates four polypeptides in the presence of 5 μ M ATP, 100 mM K⁺ and 0.005% Triton X-100 (ref. 3). To prove that a component of the acetylcholine receptor is phosphorylated by the endogenous protein kinase, phosphorylation of the acetylcholine

receptor was studied using two-dimensional immunoelectrophoresis as described by Converse and Papermaster⁴. Acetylcholine receptor-enriched membranes were incubated with γ -³²P-ATP and electrophoresed in sodium dodecylsulphate (SDS) polyacrylamide gels as described in Fig. 1. The gels were cut into longitudinal strips along the sample wells; one gel was stained for protein, another was subjected to immunoelectrophoresis at right angles into Agarose containing anti-acetylcholine receptor serum. Polypeptides derived from the acetylcholine receptor which are present in optimal concentrations form immunoprecipitates with the anti-acetylcholine receptor antibody and are recognised as 'rockets.' Figure 1b shows one major Coomassie blue stained 'rocket' which corresponds to the immunoprecipitate formed by the reaction of the antiserum with the 65,000 MW polypeptide of the acetylcholine receptor. Control gels run against pre-immune serum show no 'rockets.' Autoradiography of the dried gel could be used to determine whether the immunoelectrophoretic 'rocket' is radioactive. Figure 1c demonstrates that the same polypeptide precipitated by anti-acetylcholine receptor serum contains ³²P. Two other components of the acetylcholine receptor also react with antiserum and contain minor amounts of ³²P. Their polypeptide-antiserum concentration ratios are, however, not optimal for adequate resolution of the corresponding 'rockets.' This experiment provides firm evidence that the 65,000 MW component of acetylcholine receptor is phosphorylated by an endogenous membrane protein kinase present in receptor-enriched membranes. The major immunoprecipitate consists only of antibody against the acetylcholine receptor and the 65,000 MW component of the receptor⁴. Therefore, ³²P

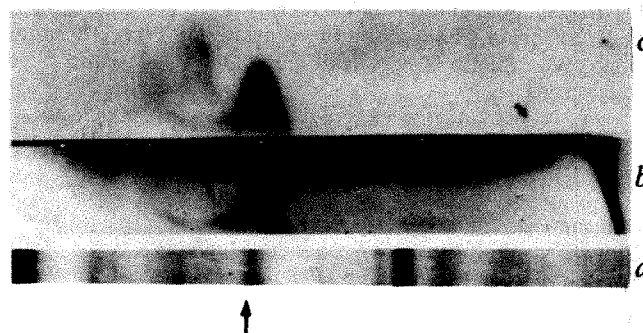


Fig. 1 Two-dimensional immunoelectrophoresis of acetylcholine receptor-enriched membranes. Acetylcholine receptor-enriched membranes were prepared from the electric organ of *T. californica* as previously described³. Membranes were incubated for 0.5 min at 0 °C in γ -³²P-ATP (1-2 μ Ci per tube), 0.25 mM EGTA, 10 mM MgAc₂, 0.0625 M Tris-HCl pH 6.8 and 100 mM KCl. The reaction was stopped by the addition of SDS to a final concentration of 4% and mercaptoethanol was added to a final concentration of 5%. Electrophoresis of 50 μ g and 75 μ g of membrane protein in SDS polyacrylamide slabs was carried out according to the method of Laemmli⁵ with 8% acrylamide in the running gel and 3% in the stacking gel. The slabs were run overnight at 8 mA per gel. The gels were then cut into longitudinal strips. *a*, The strip containing 50 μ g of membrane protein was simultaneously fixed and stained for protein for 2 h in 25% isopropanol, 10% acetic acid and 0.2% Coomassie blue and destained overnight in 25% isopropanol, 10% acetic acid. *b*, *c*, The strip containing 75 μ g of membrane protein was electrophoresed at right angles to the original direction into two layers of Agarose⁴, the first containing 1.5% Lubrol PX (Sigma) and the second containing 16% goat anti-acetylcholine receptor antiserum. Antiserum was prepared against purified *T. californica* receptor as previously described³. Immunoelectrophoresis was run at a constant voltage of 53 V (measured across the gel) for 2 h at 4 °C. The acrylamide strip and the Agarose layer were then removed to minimise electroosmotic effects. Agarose containing buffer⁴ was used to replace the removed layers. Immunoelectrophoresis was resumed for an additional 4 h at 53 V. The Agarose gels were then incubated overnight to remove unreacted serum, in 10 mM NaPO₄ pH 7.4, containing 150 mM NaCl, dried and stained and destained as described above (*b*). The gels were subjected to autoradiography on Kodak NS2T X-ray film for 5 weeks (*c*). Arrow represents MW of 65,000.

present in this 'rocket' must be derived from a phosphorylated component of the acetylcholine receptor.

In summary, we have found that the acetylcholine receptor is a substrate for an endogenous membrane protein kinase. We have recently demonstrated that phosphorylation of receptor-enriched membranes is regulated by cholinergic ligands³. Moreover, phosphorylation of the receptor may account for the observation that the acetylcholine receptor exists in two forms with different isoelectric points^{5,6}. Taken together these findings suggest that phosphorylation of the receptor may play an important role in post-synaptic events. Rhodopsin also is an extensively purified membrane protein which undergoes phosphorylation regulated by its physiological stimulus; this molecule is phosphorylated in the presence of light and ATP^{7,8}. It has been suggested^{7,8} that phosphorylation of rhodopsin might be related to a decrease in Ca²⁺ ion permeability. Furthermore, it has been shown that light-stimulated phosphorylation of rhodopsin is due to a direct conformational change of the visual pigment and not due to an effect of light on protein kinase activity. By analogy, changes in the phosphorylation of the acetylcholine receptor might be the result of conformational changes in the receptor protein induced by cholinergic ligands³. Phosphorylation could have a role in regulation of ion permeability or in other postsynaptic phenomena such as desensitisation, maintenance of the receptor at the synapse, trophic effects of innervation, and appearance of extra-junctional receptors after denervation. The finding that the acetylcholine receptor itself is phosphorylated provides a new avenue for investigation of these possibilities.

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In vitro phosphorylation of the acetylcholine receptor

In a study of the properties of the acetylcholine receptor protein (AChR) solubilised from extrasynaptic and subsynaptic areas of rat diaphragm, Brookes and Hall¹ reported differences in the behaviour of these receptors on isoelectric focusing and concluded that extrasynaptic and subsynaptic receptors "have similar properties but are still distinct molecules". Applying the same technique to crude detergent extracts of membrane fragments from *Electrophorus electricus* electric organ², we also found two forms of AChR with isoelectric points similar to those reported by Brookes and Hall. Furthermore a catalytic inter-conversion between these two forms was achieved by incubating *in vitro* the membrane extract in the presence of 100 mM NaCl or 100 mM NaF. This finding was interpreted as resulting from a phosphorylation-

dephosphorylation of the AChR. In agreement with this interpretation, it has been shown³ that endogenous protein kinase and phosphoprotein phosphatase activities are indeed present in membrane fragments isolated from the electric organ of *E. electricus*. We report here that in detergent extracts of these membrane fragments, a phosphorylation of AChR takes place when incubated in the presence of γ -³²P-ATP, Mn²⁺ and the endogenous protein kinases.

Membrane fragments were prepared by homogenising fresh or frozen electric tissue (40 g) into 80 ml of PNM (10⁻⁴M phenylmethylsulphonylfluoride, 0.02% NaN₃, 0.014 M 2-mercaptoethanol in H₂O, pH 6.2) for 5 min at 0 °C using a Virtis blender at medium speed. This homogenate was centrifuged for 30 min, 20,000g at 4 °C and the pellet resuspended in 20 ml PNM. The extraction of AChR was carried out by gently stirring the resuspended membrane fragments in the presence of 5% Triton X-100 for 60 min at room temperature. The suspension was then centrifuged at 100,000g for 60 min, at 4 °C and the supernatant ('detergent extract') collected and kept at 4 °C. The AChR was assayed with ³H- α -toxin (12.4 Ci mmol⁻¹) from *N. nigricollis*⁴; 80-100 pmol of AChR were present per ml of detergent extract. Phosphorylation of AChR was carried out by incubating the detergent extract for 30 min at room temperature in the presence of 100 mM Tris, 10⁻³M ouabain, 10 mM MnCl₂, 100 mM NaF and 4 μ M γ -³²P-ATP (16 Ci mmol⁻¹ purchased from Amersham) at pH 7.4 in a total volume of 1 ml. This mixture was then applied on top of a Sephadex G-50 column (46 cm $\times$ 2.2 cm) equilibrated and eluted in 20 mM Tris, 0.1% Triton X-100, 50 mM NaF, 10⁻⁴M phenylmethylsulphonylfluoride, 0.02% NaN₃ at pH 7.4. The protein fraction eluted in the void volume was pooled into a dialysis bag and concentrated at room temperature over polyethyleneglycol powder. The content of the dialysis bag was then removed, centrifuged for 3 min at 2,000g 4 °C and the supernatant ('phosphory-

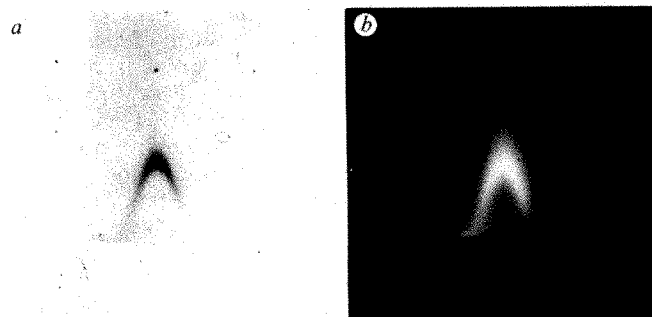


Fig. 1 Crossed-immunoelectrophoresis was carried out on Agarose gel. 15 ml of a 1% solution of Indubiose A45 in 24.5 mM veronal, 0.48 mM calcium lactate, 2 mM NaN₃, 1% Triton X-100, 73 mM Tris-HCl, pH 8.6, were poured on a 10 cm $\times$ 8.5 cm glass plate. After congelation, a 3 cm $\times$ 3 cm gel slab was then cut out in the middle of the plate and replaced with 1.6 ml of the above gel solution containing 25-50 μ l of anti-AChR serum. A 3 mm $\times$ 6 mm well is then punched in the lower left of the antibodies containing gel for the application of 10-30 μ l of antigen solution. The electrophoresis in the first dimension is carried out at 15 °C for 45 min at 10 V cm⁻¹ in the above Tris-veronal buffer pH 8.6. Anode to the right. The second dimension is carried out at 15 °C for 16-20 h at 2 volts cm⁻¹ with the anode at the top. After electrophoresis, the gel is pressed under several layers of blotting paper and a weight of about 10 g cm⁻², washed several times in 20 mM Tris-HCl, 0.1 M NaCl, 0.1% Triton X-100, pH 7.4, pressed again and finally washed in H₂O and dried. The dry gel is stained with 0.5% Coomassie blue in ethanol-acetic acid-H₂O (4.5/1/4.5) and destained in ethanol-acetic acid-H₂O (4.5/1/4.5). The destained gel is washed in H₂O and dried. It is then placed in contact with a Kodak Kodirex film. The autoradiogram is developed 1-2 d later. *a*, Coomassie blue staining of the precipitation line(s) between anti-AChR antibodies and ¹²⁵I- α -bungarotoxin-AChR complex. 20 μ l of detergent extract containing 1.8 pmol AChR and 1.5 pmol of ¹²⁵I- α -bungarotoxin (238 Ci mmol⁻¹) were applied. Anti-AChR antiserum was at a density of 5 μ l of serum per cm². *b*, autoradiograph of (*a*) after 24-h exposure.

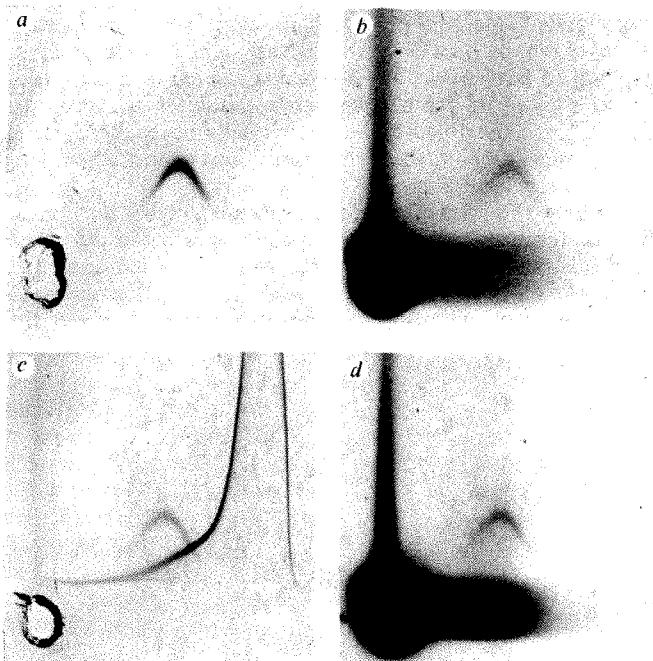


Fig. 2 Crossed-immunoelectrophoresis. Conditions as described in Fig. 1. *a*, Anti-AChR antibodies and phosphorylated detergent extract. 1.1 pmol of AChR in 18 μ l were applied. Anti-AChR antiserum was at a density of 4.5 μ l of serum per cm^2 . *b*, Autoradiograph of (*a*) after 24-h exposure. *c*, Anti-bovine serum albumin antibodies and bovine serum albumin (large peak) and anti-AChR antibodies and phosphorylated detergent extract. 1.3 pmol of AChR and 15 pmol bovine serum albumin were applied in 20 μ l. Anti-AChR and anti-bovine serum albumin antisera were respectively at a density of 3.3 and 0.55 μ l of serum per cm^2 . *d*, Autoradiograph of (*c*) after 48-h exposure.

lated detergent extract') was removed and kept at 4 °C. Rabbit sera (anti-AChR) were raised against Triton X-100 extracted AChR purified from *E. electricus* membrane fragments by affinity chromatography³. The purified AChR was analysed by dodecylsulphate polyacrylamide gel electrophoresis and found to be composed of three polypeptides of molecular weights of 43,000, 49,000 and 59,000.

To demonstrate the phosphorylation of the receptor protein, crossed-immunoelectrophoresis⁶ was used to identify and separate the AChR from the bulk of the proteins and lipids present in the detergent extract. The anti-AChR antiserum was first assayed against an aliquot of detergent extract preincubated with ¹²⁵I- α -bungarotoxin (238 Ci mmol^{-1}). Only one precipitation line was observed both by Coomassie blue staining and by autoradiography (Fig. 1*a, b*). When the phosphorylated detergent extract underwent crossed-immunoelectrophoresis in a 1% Agarose gel containing anti-AChR antibodies, again only one precipitation line was seen both by Coomassie blue staining and by autoradiography (Fig. 2*a, b*). A nonspecific absorption of phosphorylated proteins on the AChR-anti AChR precipitate could, however, be the cause of the line seen by autoradiography (Fig. 2*b*). To test this possibility, a mixture of 'phosphorylated detergent extract' and bovine serum albumin was submitted to crossed-immunoelectrophoresis in a gel containing both anti-AChR and anti-bovine serum albumin antibodies. This time, two precipitation lines were stained by Coomassie blue: one corresponded to the AChR, the other to bovine serum albumin. Only the line characteristic of the AChR was detected by autoradiography, however, (Fig. 2*c, d*), indicating without ambiguity that the AChR had been phosphorylated.

To determine which polypeptide chains of the purified AChR had been phosphorylated, AChR-anti-AChR precipitates obtained from crossed immunoelectrophoresis were cut out of agar-coated plates, solubilised in 2% sodium dodecylsulphate, 3% mercaptoethanol, 0.062 M Tris at

pH 6.8, and analysed by dodecylsulphate polyacrylamide gel electrophoresis and autoradiography. Only one polypeptide of apparent molecular weight 48,000 could be clearly detected on the densitogram obtained after scanning of the autoradiograph (Fig. 3).

The AChR protein purified from *E. electricus* gives two (ref. 5) or three (ref. 7 and A.S., unpublished) bands by polyacrylamide gel electrophoresis in sodium dodecylsulphate but only one of them corresponding to a polypeptide chain of apparent molecular weight $43,000 \pm 3,000$ is labelled by ³H-MBTA⁷ or ³H-MPTA (A.S., unpublished), two covalent affinity labels of the acetylcholine receptor site. Interestingly, it is not this chain which is phosphorylated *in vitro* but that of apparent molecular weight $48,000 \pm 3,000$.

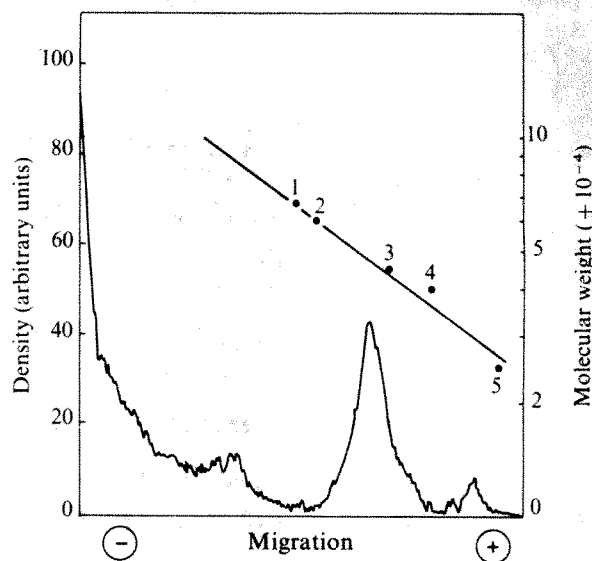


Fig. 3 Densitogram obtained from the autoradiograph of precipitates between anti-AChR antibodies and phosphorylated detergent extract after sodium dodecylsulphate polyacrylamide gel electrophoresis. Precipitates from several immunoelectrophoresis were cut out from dried Agarose plates after Coomassie blue staining and solubilised in a buffer containing 0.062 M Tris-HCl, 2% sodium dodecylsulphate, 3% 2-mercaptoethanol at pH 6.8. Electrophoresis was carried out on a 10% acrylamide slab gel of 1.5-mm width following the method of Laemmli¹¹ modified by Anderson and Gesteland¹². The gel was stained for protein with Coomassie blue, destained, washed in H_2O , dried and placed in contact with Kodak Kodirex film. The film was developed after 120 h. The densitometric scanning of the autoradiograph was carried out using a Vernon scanner and the optical density was expressed in arbitrary units. The following proteins were used as markers for molecular weight determination; 1, bovine serum albumin; 2, catalase; 3, albumin; 4, aldolase; 5, chymotrypsinogen. The band of 48,000 molecular weight could not be detected by Coomassie blue staining.

As the *in vitro* phosphorylation-dephosphorylation of the AChR takes place under conditions close to those needed for the interconversion between the two 'isoelectric' forms of the AChR, one may relate these reactions to at least two different regulatory mechanisms. (1) For instance, the 48,000 molecular weight polypeptide is the ionophore and its transport properties are regulated by phosphorylation-dephosphorylation reactions as proposed by Greengard⁹. The differences in isoelectric points between extra- and subsynaptic receptors would simply result from the fact that only the subsynaptic AChR is activated by the neurotransmitter and therefore phosphorylated. (2) In relation with the theory on selective stabilisation of developing synapses¹⁰, the phosphorylation of the AChR could be an element of the complex 'stabilisation reaction' postulated to cause the interconversion of the labile and mobile form of the AChR present in extrasynaptic areas into its stable and immobile form in the mature subsynaptic membrane. If this reaction was indeed taking place in *E. electricus* electric organ despite the fact that it is not an embryonic

system, then the phosphorylated 48,000 molecular weight chain would not necessarily represent the ionophore but could for instance be involved in the aggregation of the receptor oligomer and/or its protection against proteolysis.

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Association of DNA synthesis with the nuclear membrane in sea urchin embryos

THERE is considerable evidence implicating the cell membrane as the site for DNA replication in prokaryotic systems^{1–3}. Several laboratories have considered the possibility that the nuclear membrane may serve a similar role in eukaryotes. This model has been supported by biochemical studies in various eukaryotic cell systems^{4–8}. But, autoradiographic analyses in mammalian cells, that previously indicated that DNA synthesis was initiated at the nuclear membrane^{9,10}, now provide the strongest evidence against an involvement of the nuclear membrane in DNA synthesis^{11–13}. The sea urchin system presents several advantages for studies of DNA synthesis. On fertilisation a population of dormant unfertilised eggs begins a series of very rapid cellular divisions, the first three of which are naturally synchronous. Biochemical studies with this system have suggested that rapid replication of DNA occurs at a nuclear membrane site and that DNA may be associated with the site only during the period of DNA synthesis¹⁴. We therefore decided to undertake an autoradiographic study in the sea urchin embryo. The results presented here strongly support the role of the nuclear membrane in the synthesis of DNA.

We examined DNA synthesis during the S-phase immediately following first cleavage. In agreement with Hinegardner *et al.*¹⁵, we found that DNA synthesis begins in mid-telophase, a stage in which each chromosome is encased in its own double membrane vesicle, termed a karyomere (Fig. 1a). The grain distribution in embryos pulsed at this time for either 30 or 60 s showed a peripheral labelling pattern within each karyomere (Fig. 1a, b). A quite different distribution of grains was obtained if, instead of a short exposure to thymidine, the embryos were incubated for a longer period. After 5 min of labelling (which in this system is more than $\frac{1}{3}$ of the S-period), there was an increased number of grains in the central area of the karyomere (Fig. 1c). Although there was still dense incorporation at the periphery, the result demonstrates that chromatin is not confined to the periphery.

It is important to make certain that the grains represent incorporation of labelled nucleotide into DNA. Thymidine is

incorporated into acid precipitable material which is resistant to digestion in alkali, and is DNase labile. Also embryos exposed to label before the S-phase, and even into early telophase, showed no radioactive incorporation when assayed by autoradiography. Finally, there is virtually no background of grains appearing in the cytoplasmic areas even after long periods of incorporation.

Figure 1 shows that increased exposure to label during the S-phase results in an increase in grains appearing in the centre of the karyomere and subsequent nuclei. This indicates that either (1) the location of DNA relative to the membrane is stable and the replication machinery begins synthesis at the

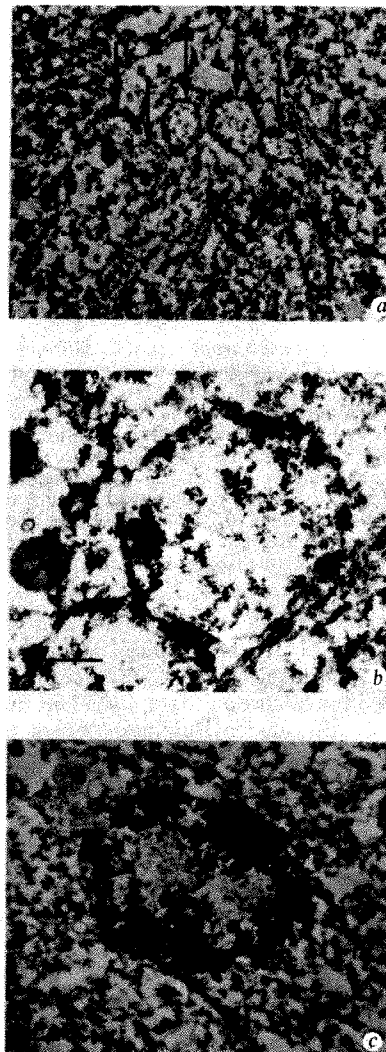


Fig. 1 Labelling of karyomeres for a, 30 s; b, 90 s, and c, 5 min following the start of S phase. Gametes from the Pacific sea urchin, *Strongylocentrotus purpuratus* were obtained by injecting 0.55 M KCl into the coelomic cavity to induce shedding. The eggs were washed, fertilised, and incubated at 16 °C as previously described¹⁴. At 100 min after fertilisation (the start of the second DNA synthetic period¹⁵) radioactive thymidine (methyl- ^3H ; New England Nuclear; 50 Ci mmol⁻¹) was added to a final concentration of 5 $\mu\text{Ci ml}^{-1}$. Samples were removed at the times indicated and the pulse stopped by the addition of an equal volume of Karnofsky's fixative. After settling, the labelled embryos were resuspended in fresh fixative, allowed to sit for 2 h, and postfixed for an additional hour in 1% osmium tetroxide. Following ethanol dehydration, the fixed embryos were embedded in Epon resin and sectioned on an LKB ultramicrotome. Purple/gold sections were removed from the air/water interface using Formvar coated copper grids and stained with uranyl acetate and lead citrate, then carbon coated under vacuum. The grids were coated with Ilford L-4 emulsion (Polysciences) using the technique of Stevens²⁴ and stored at -60 °C for 14 d–6 months. After development, the grids were routinely restained with lead citrate. Micrographs were made using a Philips 300 electron microscope. The bar in each figure represents 1 μm .

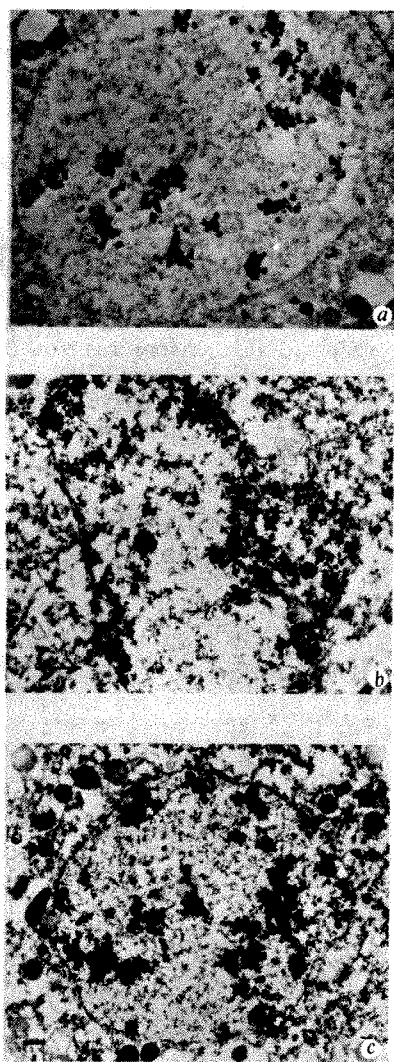


Fig. 2 Distribution of grains in nuclei labelled for various periods of time. *a*, Nucleus of embryo labelled continuously for 20 min beginning at 100 min after fertilisation. *b*, Embryos pulsed for 30 s, washed twice in normal sea water, and then resuspended in normal sea water containing 200 μ M unlabelled thymidine. The chase period was for 4.5 min. *c*, Embryos pulsed for 30 s and chased for 20 min in cold thymidine as in *b*. Embryos were labelled with ^{3} H-thymidine, fixed, and prepared for autoradiography as described in Fig. 1.

periphery and moves into the central area, or (2) nascent DNA is displaced from a stable site of replication at the periphery and moves into the centre. To distinguish between these two possibilities we looked at the distribution of continuously labelled DNA after the completion of the S-phase and the fate of the DNA synthesised during early S using a pulse-chase analysis. A typical result of the first study is shown in Fig. 2*a*. The pattern of grains seen in nuclei after 20 min of labelling indicates a concentration of radioactive DNA within the central areas and little or none present at the periphery (Fig. 2*a*). Under such conditions the DNA is uniformly labelled and the embryos are no longer in the S-phase. This shows that once replication is completed, virtually all of the DNA is displaced away from the membrane region; and that most of the DNA is not permanently associated with the membrane. A pulse-chase analysis supports these conclusions. Nuclei of embryos pulsed during the first 30 s of the S-phase and chased with cold thymidine for either 4.5 min or 19.5 min are compared in Fig. 2*b* and *c*, respectively. After a short chase, karyomeres typically showed a trend towards more homogeneous labelling. While there is still a large percentage of grains present at the periphery, there appears to be a significant increase in central label. This trend in apparent relocalisation of grains is much clearer in Fig. 2*c* which shows that after a long chase period the DNA

labelled during a short pulse in early S is located predominantly in the central region of the nucleus and does not maintain a stable location at the periphery. Taken together, these results support the second possibility presented above: once replication is completed, the nascent DNA is displaced away from a stable site of replication located at the nuclear membrane.

We attempted to quantify our results using a histogram analysis (defined in legend of Fig. 3) as used by Huberman *et al.*¹². Figure 3 compares histograms derived from embryos labelled for pulse, long term, and pulse-chase periods. All the embryos labelled for short periods have central activities of less than 1.0 (Fig. 3*a*). The mean central activity in these embryos is 0.4 indicating that the peripheral areas are enriched with many more grains than would be expected if the nascent DNA were labelled homogeneously in all areas of the karyomere. In contrast, Fig. 3*b* shows that the majority of nuclei labelled continuously through the complete S-phase have central activities greater than 1.0 indicating a general lack of peripheral label. This result also indicates that most of the DNA is located in the central region of the nucleus. When embryos are pulsed

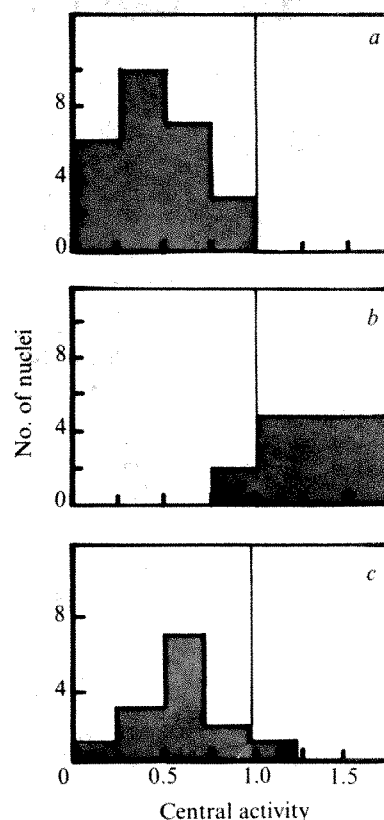


Fig. 3 Distribution of grains in nuclei of sea urchin embryos after various labelling conditions. Histogram analysis of central activities in cells labelled as in Fig. 2 for: *a*, pulse (30–100 s); *b*, continuously labelled (20 min); *c*, pulse/chase (labelled 30 s then chased for 4.5 min). Central activity is defined as the ratio of the fraction of grains which are central to the fraction of area which is central. A central activity of 1.0 indicates a homogeneous distribution of grains throughout the nucleus; ratios of < 1.0 signify a preferential labelling of the peripheral area. A central activity of > 1.0 indicates a preferential localisation of grains away from the periphery. Only karyomeres/nuclei with four or more grains were scored. Grains outside the nuclear membrane, regardless of their proximity, were not counted. For karyomeres, a rim 0.5 μ m in width was considered as the peripheral area. For nuclei, the peripheral area was a rim 1.5 μ m in width. This differentiation in width was made so that the percentage of area considered central in all cases remained approximately the same. To determine the ratio of area, paper tracings of the micrographs were made, the peripheral area measured and cut, and the fraction determined by the weight of the paper. The paper rim was then placed on the micrograph to determine the fraction of grains that were central. After the 4.5 min chase in (*c*) many grains are associated with the periphery probably because the large internal nucleotide pools¹⁶ are not completely diluted during such a short chase.

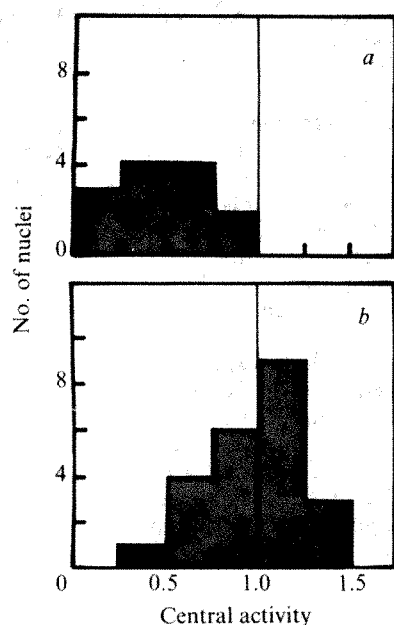


Fig. 4 Histogram analysis of grain distribution in late S phase labelled nuclei. *a*, Nuclei labelled for 30 s. *b*, Nuclei labelled for 90 s. Embryos developing at 16 °C were labelled beginning at both 108 and 112 min after fertilisation. After radioactive precursor incubation, samples were removed, fixed, and prepared for autoradiography as described in Fig. 1. Results from the short and long term labelling at both the 108 and 112 min time points were virtually the same and were combined.

and then chased for a short period the values for the central activities are shifted toward 1.0 (Fig. 3c), a trend away from the strong peripheral labelling and towards a more homogeneous labelling pattern.

Finally, we tested whether nascent DNA synthesised in the late S-phase is also localised in the peripheral region (Fig. 4). Embryos at 110 min after fertilisation were pulsed for 30 or 60 s. The S-phase in these embryos is at least two-thirds completed and the nuclei are entirely reformed. At this time the incorporation of exogenous precursor seems to occur much more slowly, based on the number of grains detected per nucleus in comparison to embryos in the early S-phase. But, like the patterns in early S-phase, the distribution of grains in nuclei pulse-labelled during late S indicates that incorporation is still taking place largely at the periphery (Fig. 4a). When these embryos are allowed to incorporate label for longer periods the central activity values are distributed around 1.0 (Fig. 4b) indicating a shift of grains away from the membrane.

The autoradiographic evidence presented here supports our previous biochemical studies indicating that the nuclear membrane may be involved with the temporal regulation of DNA replication in the sea urchin¹⁴. An enrichment of radioactive DNA in a presumptive DNA-membrane fraction was also found when we examined pulse-labelled nuclei of cultured mouse fibroblasts (3T3 cells)¹⁷; however an autoradiographic study in this system has not been reported. It is difficult to resolve our results with the autoradiographic results of others using mammalian culture cells. A possible explanation may be that autoradiographic analysis cannot distinguish between replication and repair synthesis of DNA, two activities known to occur in the S-phase nucleus. In the naturally synchronous sea urchin embryo where, during the S phase 1.8 pg of DNA is replicated in 12–15 min (ref. 15) semi-conservative replication may be assumed to be the principal synthetic activity. In mammalian culture cells, the period of time during which DNA is replicated is relatively long and it is now thought that replication events are initiated sporadically during this time¹⁸. This suggests that replication is not a continuous event during the DNA synthetic period and may be characterised by many starts and stops^{19,20}. Thus, a pulse label of cells from either

synchronised or unsynchronised cultures may result in the incorporation of exogenous precursor due to both repair and replication. Such a situation would confuse the interpretation of autoradiographs in determining subnuclear sites of replication.

In mammalian systems there is general agreement that during the late stage of the S-phase DNA synthesis occurs preferentially near the membrane^{9,11,12}. This result is commonly considered to be due to the replication of heterochromatin which is known to be replicated late in the S-phase^{21,22} and to be located near the membrane^{11,12,23}. In our studies the pulse-labelled DNA is preferentially located at the membrane both in the early and late S-phase. Furthermore it is clear that in this system virtually all of the DNA is located in the central region of the nucleus (Figs 2a, 3b). The simplest conclusion is that DNA synthesis occurs at the nuclear membrane and is relocated away from the membrane.

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Primary structure of porcine relaxin: homology with insulin and related growth factors

HISAW and co-workers^{1,2} first described a factor from sow corpora lutea that caused relaxation of the pubic symphysis in oestrogen-primed guinea pigs. Subsequent studies have shown that the active substance, termed relaxin, is a low molecular weight peptide hormone. Its presence has been demonstrated during pregnancy in female pigs, guinea pigs, rabbits, mice and rats^{3,4}, and, more recently, in humans⁵. Its actions include dilatation and softening of the cervix, inhibition of uterine contractions and relaxation of the pubic symphysis and other pelvic joints. Relaxin, which has a molecular weight of approximately 6,000, consists of two non-identical chains joined by disulphide bridges⁶. We have now determined, and report here, the complete amino acid sequence of porcine relaxin.

Relaxin was purified from pregnant sow ovaries⁶; biological activity was localised by the mouse pubic symphysis bioassay⁷. Relaxin fractions used for structural studies had biopotencies in the range 1,100–1,500 guinea pig units (GPU) per mg relative to reference preparation NIH-R-P1 (potency 442 GPU mg⁻¹).

As a preliminary to sequence analysis, relaxin was reduced with dithioerythritol in urea or guanidine solutions

A CHAIN

H - Arg - Met - Thr - Leu - Ser - Glu - Lys - Cys - Cys - Gln - Val - Gly -
Cys - Ile - Arg - Lys - Asp - Ile - Ala - Arg - Leu - Cys - OH

Fig. 1 Amino acid sequences of A and B chains of relaxin. PCA, pyrrolidone carboxylic acid.

B CHAIN

PCA - Ser - Thr - Asn - Asp - Phe - Ile - Lys - Ala - Cys - Gly - Arg - Glu -
Leu - Val - Arg - Leu - Trp - Val - Glu - Ile - Cys - Gly - Ser - Val - Ser -
Thr - Trp - Gly - Arg - OH

and alkylated with ^{14}C iodoacetic acid⁸. A number of methods were investigated for chain separation. During attempts to use ion exchange chromatography for this purpose it was observed that the longer chain (30 amino acids, designated 'B') was insoluble in the pH range 4-7, whereas the shorter chain (22 amino acids, designated 'A') remained soluble. Thus chain separation could be achieved simply by low speed centrifugation of the reduced carboxymethylated preparation. Schwabe *et al.* have described a similar procedure⁹.

The amino acid sequence of the A chain (Fig. 1) was established in three separate experiments by manual Edman degradation¹⁰. After 21 cycles the residual sample was subjected to amino acid analysis. Free carboxymethylcysteine was identified; thus this residue occupies the C terminal position in the A chain.

Sequence analysis of the B chain proved more difficult since the N terminus was found to be resistant to Edman degradation. Removal of the N-terminal blocking group could be achieved, however, by a pyrrolidone carboxylate peptidase¹¹. Thus the terminal residue was identified as pyroglutamic acid. Edman degradation was then carried out for 16 cycles on the deblocked B chain.

The remainder of the sequence (Fig. 1) was established by manual Edman degradation of the peptide Leu¹⁷ . . . Arg³⁰ isolated from a tryptic digest of carboxymethylated B chain. Confirmatory studies were carried out through Edman degradation and carboxypeptidase digestion of tryptic peptides isolated by preparative paper electrophoresis. Schwabe *et al.*⁹ have previously proposed a sequence for the A chain of relaxin identical to ours except that they find residue 10 to be glutamic acid whereas in three different preparations we clearly identified glutamine at this position. Deamidation of their material during isolation or sequence analysis could explain this discrepancy.

There are several features of the relaxin structure that deserve comment. The marked structural similarity to insulin is evident. Thus, relaxin consists of two dissimilar peptide chains of 22 and 30 residues respectively. The number and distribution of half-cystine residues are identical in the two hormones. Neither has free sulphhydryl groups. We have not yet rigorously established the pairing of the three disulphide bonds in relaxin; however, preliminary evidence suggests that the arrangement is identical to that of insulin, with two interchain bridges and an intra-

chain bridge in the relaxin. A chain linking the half-cystines at positions 8 and 13.

Amino acid sequence homology between relaxin and insulin is more obvious in the B than in the A chains (Figs 2 and 3). Superficially the A chain sequence homology does not seem impressive apart from the exact alignment of the four half-cystine residues. But, reference to the three-dimensional structure of insulin known from X-ray crystallographic studies^{12,13} shows that there is virtually complete conservation of the residues in the insulin A chain that contribute to the hydrophobic core of the molecule. These are the half-cystine residues found in insulin at A6 and A11, together with the A2 isoleucine (leucine in relaxin) and A16 leucine (isoleucine in relaxin). Two further residues from the insulin B chain (B11 and B15 leucines) complete the hydrophobic core; both are conserved in relaxin. A further perspective appears when residues invariant in all known insulin structures¹³ are examined. These (designated with asterisks in Figs 2 and 3) comprise 10 residues in each chain. At these 20 positions, a total of 15 residues are either identical or conservatively substituted in relaxin. Within the central helical region of the insulin B chain (B9 to 19) four of the five invariant residues are identical in relaxin; the fifth (tyrosine B16) is replaced by tryptophan in relaxin. At several other positions within this sequence substitutions are seen which conserve the hydrophilic or hydrophobic nature of the residue (for example, serine B9 is replaced by arginine in relaxin; glutamic acid B13 by arginine; leucine B17 by valine; valine B18 by isoleucine). These findings suggest that important elements of the tertiary structure of insulin are also present in relaxin, and that their overall three-dimensional structures may be quite similar.

An important difference is seen in the B chain where the zinc-binding histidine residue found at position B10 in almost all insulins is replaced by glutamic acid in relaxin. This makes it most probable that relaxin will not bind zinc and thus will not be able to form zinc-coordinated dimers or hexamers. In this relaxin resembles guinea pig, coypu and hagfish insulins, which are stable as monomers in solution even at high concentrations.

Relaxin has arginyl residues at the N terminus of the A chain (A1) and the C terminus of the B chain (B30). This arrangement, taken with the other evidence for homology, strongly suggests that relaxin is derived from a proinsulin-like biosynthetic precursor by proteolytic cleav-

Fig. 2 Comparison of sequences of insulin and relaxin A chains. Identical residues are enclosed in rectangles. Residues related through favoured substitutions²² are enclosed in circles. Residues invariant in all known insulin structures¹³ are indicated by asterisks.

Relaxin	Arg	Met	Thr	Leu	Ser	Glu	Lys	Cys	Cys	Gln	Val	Gly
Insulin			Gly	Ile	Val	Glu	*5 Gln	*Cys	*Cys	Thr	Ser	Ile ¹⁰
Relaxin	Cys	Ile	Arg	Lys	Asp	Ile	Ala	Arg	Leu	Cys		
Insulin	*Cys	Ser	Leu	Tyr	15 Gln	*Leu	Glu	Asn	*Tyr	*20 Cys	*Asn	

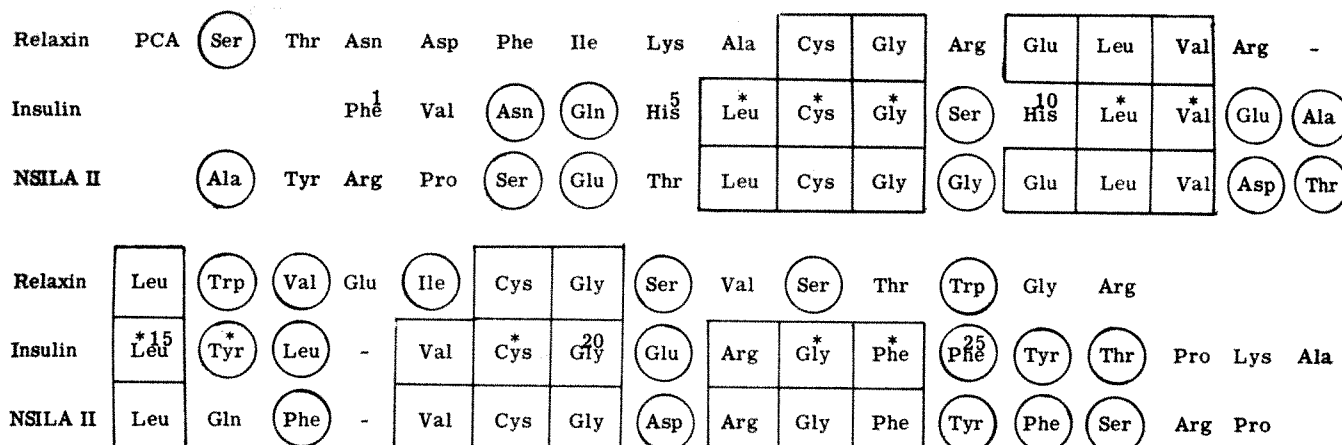


Fig. 3 Comparison of sequences of insulin and relaxin B chains and NSILA II¹⁸. Homologies and invariant residues are indicated as in Fig. 2. Gaps have been introduced in three places to improve the alignments.

age of a peptide connecting B30 to A1. Some earlier studies on relaxin also support the existence of a prohormone^{14,15}. In confirmation of earlier findings⁶ we have identified in ovarian extracts several forms of biologically active relaxin that differ slightly in net charge and amino acid composition from the peptide shown in Fig. 1. Chemical characterisation of these variants is still in progress; they could represent intermediates in the conversion of prohormone to hormone analogous to those described for insulin^{16,17} or degradation products.

The evolutionary relationship between relaxin and insulin is of considerable interest. It is likely that relaxin is related to insulin through a common ancestral polypeptide resembling proinsulin. Rinderknecht and Humbel¹⁸ have recently demonstrated a sequence homology between the insulin B chain and two polypeptides with non-suppressible insulin-like activities (NSILA I and II). These peptides are also clearly structurally related to relaxin (Fig. 3).

These homologies provide a basis for categorising relaxin as a member of a family of insulin-like growth factors with both structural and functional similarities in modulating cell growth and activity. These include nerve growth factor¹⁹, somatomedins A and C²⁰, and multiplication-stimulating factor²¹, as well as insulin and non-suppressible insulin-like activity (NSILA). Further structural studies should delineate the precise evolutionary relationships between these molecules.

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Note added in proof: Our collaborators on the X-ray crystallography of relaxin (Drs Neil Isaacs and Guy Dodson of the University of York) have been able to fit the residues between the disulphide bridges of both chains of relaxin (A8-A22 and B10-B22) into the two-zinc insulin structure without producing significant distortion. Schwabe *et al.*²³ have proposed a sequence for the relaxin B chain that differs slightly from our own at the C terminus. Whether this discrepancy is due to technical problems or to the known C terminal heterogeneity of relaxin (see text) is unclear at present.

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In vivo photoinactivation of *Escherichia coli* ribonucleotide reductase by near-ultraviolet light

NEAR-ULTRAVIOLET light (320-400 nm) is lethal to bacteria¹, yeast cells² and mammalian cells³. A large body of evidence correlates cell death and survival with the induction and repair of pyrimidine dimers in the DNA of cells irradiated by far-ultraviolet light (200-300 nm) (refs 4-7). The induction of pyrimidine dimers has also been demonstrated by 365-nm near-ultraviolet light but at 7×10^5 -fold lower efficiency than by 254-nm far-ultraviolet light⁸. With the exception of certain *Escherichia coli* strains with multiple deficiencies in DNA repair^{9,10} the small number of pyrimidine dimers produced by 365-nm near-ultraviolet light does not account for a significant fraction of the biological damage either in terms of lethality¹¹ or the inactivation of transforming DNA¹². The killing of cells by near-ultraviolet light is oxygen dependent whereas that by far-ultraviolet light is oxygen independent^{3,13}. The operational distinction between far- and near-ultraviolet light based on oxygen dependence of lethality coincides with the upper limit of ultraviolet light absorption by the

Table 1 Effect of near-ultraviolet irradiation on the activity of RDP-reductase, β -galactosidase and malate dehydrogenase

Dose (erg mm ⁻² $\times 10^{-4}$)	Activity remaining (%)		
	RDP- reductase	β - galactosidase	Malate dehydrogenase
0	100	100	100
3.0	35.4	—	—
7.5	24.7	—	—
15	3.5	99.5	97.6

E. coli TAU-bar was grown in glucose salts medium¹⁹. Mid exponential phase cultures were resuspended in 0.8% saline supplemented with 0.5 g l⁻¹ KCl and 0.4 g l⁻¹ CaCl₂ at 1.0×10^{10} cells per ml. Saline suspensions were irradiated at a dose rate of 3.0×10^3 erg min⁻¹ mm⁻² for 10, 25 and 50 min with aeration at 37 °C or were aerated at 37 °C without irradiation. RDP-reductase was assayed by the method of Warner²⁰ in whole cells made permeable to nucleotides by ether treatment. β -galactosidase and NAD-dependent malate dehydrogenase were assayed by standard spectrophotometric methods. Enzyme activities are expressed as a percentage of the unirradiated controls.

DNA at about 310 nm¹⁴ and may reflect the involvement of a primary target site other than DNA in the killing of cells by near-ultraviolet light. In *E. coli* deoxyribonucleotides are formed *de novo* by the reduction of ribonucleoside diphosphates¹⁵. Two of the components of the ribonucleoside diphosphate reductase (RDP-reductase) complex, the non-haem iron protein subunit of the RDP-reductase¹⁶ and the functionally linked flavoprotein thioredoxin reductase¹⁷ have strong absorption in the near-ultraviolet region. I show here that near-ultraviolet irradiation of *E. coli* cells selectively destroys RDP-reductase activity *in vivo* and present evidence relating the loss of RDP-reductase to the loss of cellular viability and to the inability of irradiated cells to support the replication of DNA phages.

Activity of RDP-reductase was measured in *E. coli*

Fig. 1 Percentage survival of colony forming ability as a function of near-ultraviolet dose in unsupplemented medium ($\square$), medium supplemented with deoxyadenosine ($\bullet$) or adenosine (Δ). Exponential phase cultures of *E. coli* TAU-bar were diluted to 5×10^7 cells per ml in glucose salts medium and were irradiated at 20 °C with aeration at a dose rate of 3.0×10^3 erg min⁻¹ mm⁻². In parallel experiments the medium was supplemented with deoxyadenosine or adenosine at 250 μ g ml⁻¹. All media contained thymine at 2.0 μ g ml⁻¹. Colony forming ability of irradiated cells was determined on L-agar. Irradiated glucose salts medium had no phototoxic effect when incubated with unirradiated cells.

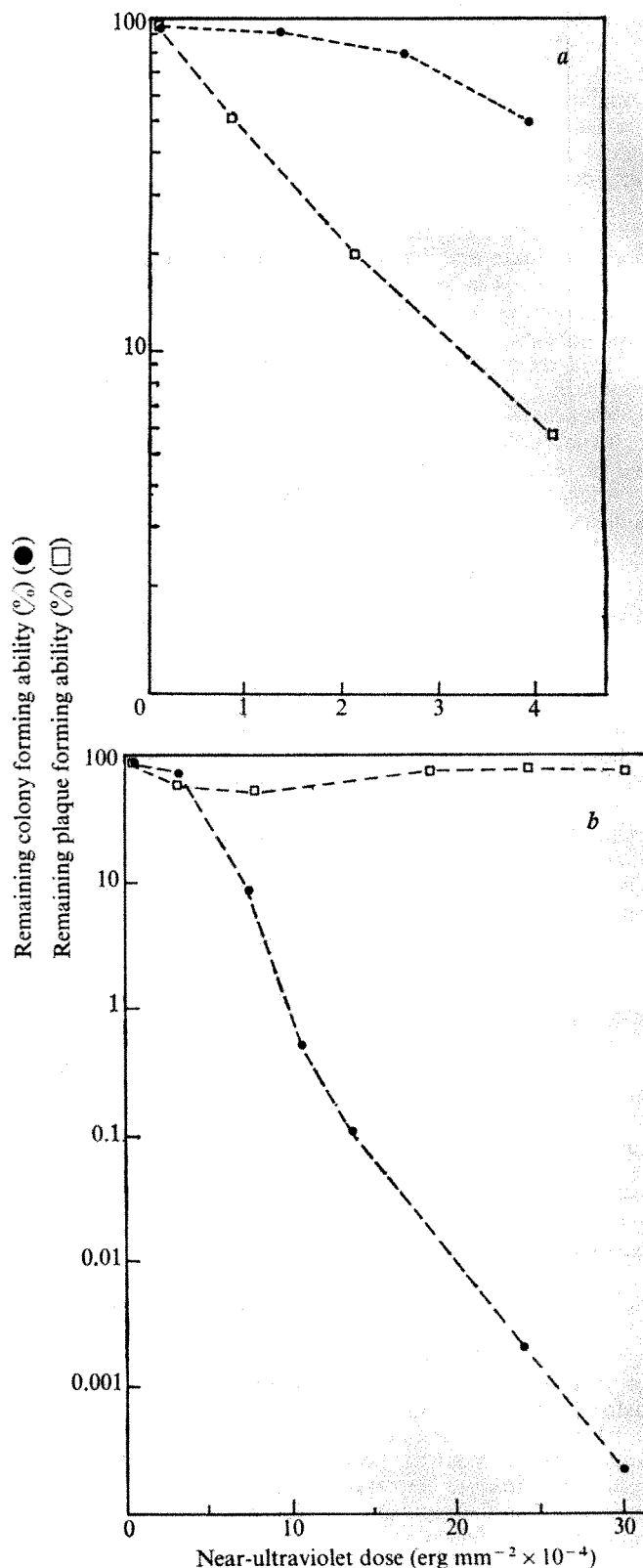
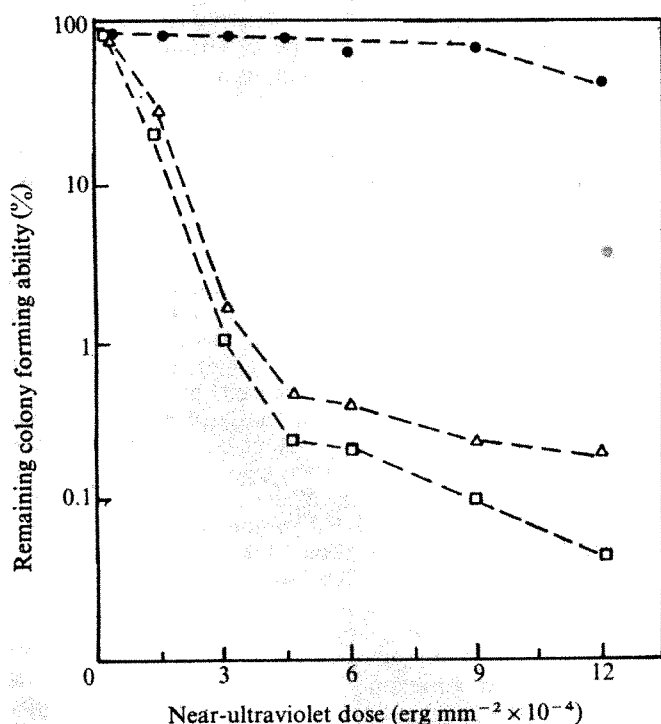


Fig. 2 Percentage of survival of colony-forming ability ($\bullet$) and plaque-forming ability ($\square$) of *E. coli* Hfr H as a function of near-ultraviolet dose with *a*, T₇ phage and *b*, MS-2 phage. Irradiation conditions were as for Table 1. Irradiated cells were infected with T₇ or MS-2 phage at a multiplicity of 0.001. After 8 min adsorption at 37 °C the number of infectious centres was determined by plating with 5×10^6 unirradiated indicator bacteria on L-agar supplemented with 0.1 g l⁻¹ MgSO₄ and 0.2 g l⁻¹ CaCl₂. The total number of plaques was corrected for the number of chloroform resistant unadsorbed phage. In parallel experiments the colony forming ability of identically irradiated but uninfected cells was determined on L-agar.

TAU-bar as a function of the near-ultraviolet dose. Philips HPW 125 W black-light lamps (maximum emission at 365 nm) were used as the near-ultraviolet source. The irradiation apparatus² and the spectral characteristics of the light source¹⁸ have been described previously. Table 1 shows that about two-thirds of the RDP-reductase activity was destroyed by a dose of 3.0×10^4 erg mm⁻² and that there was nearly complete loss of RDP-reductase activity with a dose of 1.5×10^5 erg mm⁻². The activities of β -galactosidase and malate dehydrogenase were not significantly affected in this dose range indicating that the photo-destruction of RDP-reductase activity by near-ultraviolet was a specific effect.

Deoxythymidylate may be synthesised by two independent routes in *E. coli*, either by the *de novo* pathway involving RDP-reductase and thymidylate synthetase or by the salvage pathway utilising thymine and a deoxyribosyl donor²¹. When deprived of thymine, strains lacking thymidylate synthetase undergo lethal unbalanced growth termed thymineless death²². If the photoinactivation of RDP-reductase results in a metabolic state analogous to that occurring in thymineless death, it should be possible to abolish or diminish the lethal effect of near-ultraviolet light by providing the culture with an exogenous deoxyribosyl source. This prediction was tested using *E. coli* TAU-bar, a strain which in addition to lacking thymidylate synthetase is also deficient in deoxyriboaldolase²³. The second mutation results in efficient deoxythymidylate synthesis via the salvage pathway by blocking deoxyribose catabolism^{23,24}. Figure 1 shows the survival curves of *E. coli* TAU-bar irradiated in unsupplemented medium, in medium supplemented with 250 μ g ml⁻¹ deoxyadenosine or 250 μ g ml⁻¹ adenosine. With an initial dose of 4.5×10^4 erg mm⁻² rapid loss of viability occurred and was followed by a slower rate of killing with additional irradiation in unsupplemented medium. The inclusion of deoxyadenosine in the medium prevented the killing of cells up to a dose of 9.0×10^4 erg mm⁻². The survival pattern was similar in the medium supplemented with adenosine as in the unsupplemented medium indicating that the sparing effect was specific for the deoxyribosyl moiety. A reduction of the lethal effect of near-ultraviolet light also occurred with the prototrophic strain *E. coli* B/r thy⁺. After a dose of 4.5×10^4 erg mm⁻² survival was 0.020% in the unsupplemented medium, 0.20% in the medium supplemented with 250 μ g ml⁻¹ deoxyadenosine and 4.2% in the medium supplemented with both 250 μ g ml⁻¹ deoxyadenosine and 20 μ g ml⁻¹ thymine (survival curves not shown).

Another property of cells irradiated by near-ultraviolet light is the loss of their ability to support the replication of DNA phages^{25,26}. *E. coli* cells regain the ability to support the replication of T₁ DNA phage after low doses of near-ultraviolet irradiation when incubated in broth before infection suggesting that a metabolic lesion is involved²⁵. If this lesion is the loss of the RDP-reductase, and if other requisite host functions remain sufficiently intact to produce infectious phage, then it would be expected that the replication of RNA phages would not be suppressed by near-ultraviolet irradiation of the host. This prediction was tested using *E. coli* K-12 Hfr H as the host strain and the DNA phage T₇ and the RNA phage MS-2. Since the replication of the MS-2 genome is carried out by a phage specific RNA-dependent RNA polymerase²⁷ there is no requirement for deoxyribonucleotides. The capacity of irradiated cells to support the replication of T₇ phage was lost exponentially while there was a relatively small decline in the cellular viability up to a dose of 4.8×10^4 erg mm⁻². In contrast, cells which had received doses up to 3.0×10^5 erg mm⁻² retained their ability to support the replication of MS-2 phage while cellular viability was reduced to 0.00025% (Fig. 2).

The data presented here are consistent with the interpre-

tation that the underlying event in the killing of exponentially growing *E. coli* cells by near-ultraviolet light, and in the loss of the ability of irradiated host cells to support the replication of DNA phages, is the photoinactivation of the RDP-reductase complex.

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Leader sequence of 71 nucleotides devoid of G in tobacco mosaic virus RNA

EXHAUSTIVE T₁ ribonuclease digestion of the RNA of tobacco mosaic virus (TMV) liberates an unusually large T₁ oligonucleotide (~72 nucleotides) called Ω (refs 1-4). Analysis of partial T₁ ribonuclease digestion products of uniformly ³²P-labelled TMV RNA shows that Ω is no more than 200 nucleotides from the 5'-end of the chain. We have used polynucleotide kinase to fix a radioactive phosphate to the 5'-end of both Ω and intact TMV RNA (after removal of the pppm⁷G 'cap') and determined the sequence of the first 15-16 nucleotides at the 5' terminus of each by two-dimensional homochromatography of partial P₁ nuclease digests. The results show that Ω begins only one nucleotide away from the 5'-end of the RNA chain, the terminal sequence being m⁷GpppG Ω . . . Consequently, positions 2-72 of the TMV RNA molecule are devoid of G (guanosine residues).

An early attempt to locate Ω in the RNA chain was founded upon the erroneous belief that mild sodium dodecyl sulphate treatment of TMV removes subunits preferentially from the end of the particle containing the 3' OH of the RNA chain⁵. Mandeles¹ found that Ω was exposed early in the course of such treatment, leading him to conclude that the oligonucleotide was within about 200 nucleotides of the 3' OH end. Recently, however, it has been established that the polarity of sodium dodecyl sulphate uncoating is the opposite of what was previously supposed^{6,7}, implying that Ω is near the 5' rather than the 3' end of the RNA molecule.

As noted above, TMV RNA begins with the sequence m⁷G⁺ ppp⁺G . . . (refs 8,9). The triphosphate bridge which joins the m⁷G cap to the 5' end of the RNA chain proper is resistant to endonucleolytic cleavage, and the RNase-resistant moiety, m⁷GpppGp, can be easily separated from the other products of digestion by ionophoresis at pH 3.5⁸. We have taken advantage of this property to screen a partial T₁ RNase digest of uniformly ³²P-labelled TMV

RNA for fragments coming from the 5'-end. When the partial digest was fractionated on a polyacrylamide gel, seven bands contained significant amounts of the fragments sought (Fig. 1a). Fragments 1-3 were successfully freed of contaminating material by an additional step of gel electrophoresis; fragments 4-7, however, have not yet been purified sufficiently to warrant sequence analysis.

The T1 RNase fingerprint of purified fragment 2 (240 nucleotides) is shown in Fig. 2. The cap structure, m⁷GpppGp, migrates as a diffuse spot to the right of UUG. The corresponding product in the pancreatic RNase fingerprint (not shown) was m⁷GpppGU.

As shown in Fig. 2, fragment 2 contains a large T1 oligonucleotide which we have identified as Ω , in line with the findings of Mandeles¹ (see above). Evidence that the indicated spot is indeed Ω includes the following: (1) treatment of the oligonucleotide with pancreatic RNase gives products in the ratio 4 AAU, 2AAAC, 3 AU, 6 AAC, 6 AC, approximately 13 U and 1-2 C to one G, in substantial agreement with the pancreatic RNase catalogue described for Ω by Garfin and Mandeles⁴; (2) the U₂ RNase catalogues are in reasonable accord except that we find U₅A rather than the U₆A reported by Garfin and Mandeles⁴ and probably U₄A instead of U₃A; and (3) the putative Ω has the same mobility in bidimensional gels as does authentic Ω from a T1 RNase digest of whole TMV RNA, Ω also forms

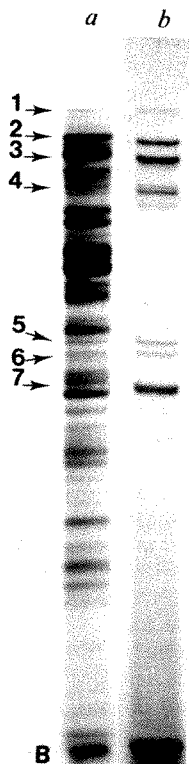


Fig. 1 Polyacrylamide gel electrophoresis of a partial T1 RNase digest of *a*, uniformly ³²P-labelled TMV RNA and *b*, 5'-³²P-labelled TMV RNA. Uniformly labelled TMV RNA was prepared as described by Guilley *et al.*¹⁷; 5'-terminal post-labelling of TMV RNA was carried out after chemical removal of m⁷G (ref. 10) and subsequent phosphatase treatment (0.02 units calf intestinal alkaline phosphatase per 100 μ g RNA; incubation for 30 min at 37°C). The buffer was 0.05 M Tris, 0.01 M MgCl₂, pH 7.5. The uncapped RNA (300 μ g in a final volume of 0.2 ml) was then incubated for 30 min at 37°C with 2 units polynucleotide kinase and 3 μ M γ -³²P-ATP (specific activity: 200 Ci mmol⁻¹). After each step of enzyme treatment in the above procedure the solution was extracted with phenol to inactivate the enzyme, and the integrity of the RNA was verified by electrophoresis in polyacrylamide-Agarose gels. Partial T1 RNase digestion was with 1 unit enzyme per 300 μ g RNA in 0.1 M Tris, 0.001 M EDTA, pH 7.5. After 30 min at 0°C the reaction was stopped by phenol extraction and the partial digest was electrophoresed through an 8% polyacrylamide gel¹⁷. Arrows mark the positions of the 5' terminal fragments referred to in the text; B marks the position of the bromophenol blue marker.

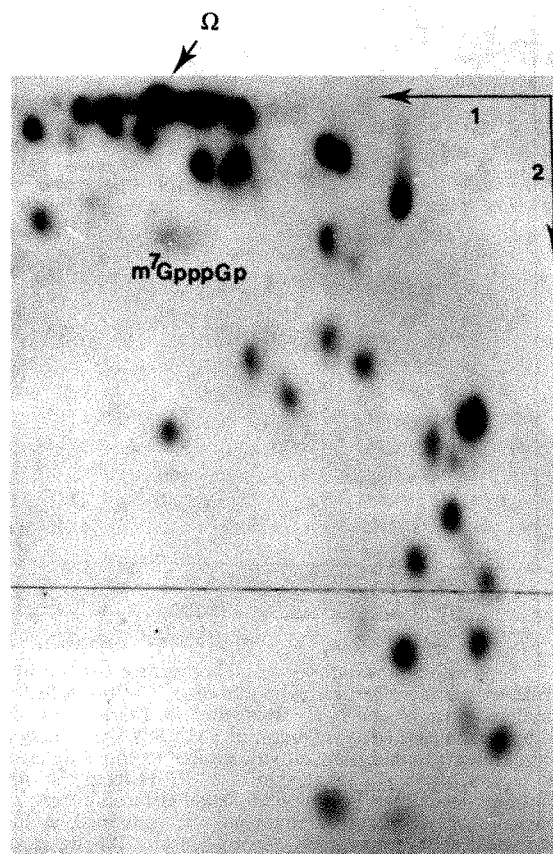


Fig. 2 T1 RNase fingerprint of 5'-terminal fragment 2 of TMV RNA. The first dimension was on a cellulose acetate strip at pH 3.5 and the second dimension on DEAE paper wetted with 7% formic acid.

part of fragment 3 (200 nucleotides) and fragment 1 (approximately 280 nucleotides).

A preliminary investigation of partial T1 RNase digestion products of purified fragment 2 suggested that Ω might lie very near the 5'-end of the sequence. This led us to determine the sequence of the first several nucleotides at the 5'-end of TMV RNA by partial P1 nuclease digestion of 5'-³²P-labelled fragments in the hope that this sequence might overlap that of Ω . But, before a radioactive phosphate can be attached at the 5'-terminus of TMV RNA, the blocking group must be removed. Towards this end, non-radioactive TMV RNA was treated with sodium periodate and with aniline to eliminate the m⁷G (refs 10,11) and the 5'-triphosphate group was removed with alkaline phosphatase. A radioactive phosphate was then fixed to the newly exposed 5' OH group by treatment with polynucleotide kinase prepared from T4-infected *E. coli*¹² in the presence of γ -³²P-ATP.

The 5'-³²P-TMV RNA was partially digested with T1 RNase and the mixture of fragments fractionated by polyacrylamide gel electrophoresis (Fig. 1b). As expected, the seven principal radioactive bands, each of which should represent a fragment from the 5'-end of the TMV RNA chain, correspond closely in mobility with the seven capped fragments detected by screening the partial digest of uniformly labelled RNA for m⁷GpppGp (see Fig. 1a).

The 5'-terminally labelled fragments shown in Fig. 1b were digested with the non-specific endonuclease P1 from *Penicillium citrinum* in mild conditions in order to generate a random population of 5'-labelled partial degradation products¹³. Figure 3a shows an autoradiogram of such a digest analysed by two dimensional homochromatography¹⁴. All seven fragments gave identical patterns. The first dimension of the fractionation was by electrophoresis at pH 3.5 (separation according to charge) and the second dimension by homochromatography (fractionation according to size). Within the limits of resolution in the second dimension, the

18–36 nucleotides. The fragment migrated in the gel with a mobility similar to that of the 30-nucleotide long 2S rRNA from *D. melanogaster*¹⁴.

To determine if the binding is sequence specific, the RNA fragment was eluted from the gel¹⁵ and RNase T1 and pancreatic RNase digests were subjected to fingerprint analysis¹⁶ (Fig. 2). Both fingerprints yielded simple patterns, the number of intense spots agreeing roughly with the expected size of the fragment. This suggested that the binding of IF3 was not random, but sequence specific, although the presence of more than one binding site could not be excluded.

The structures of the main oligonucleotides in the digests are shown in Fig. 3a. Four oligonucleotides in the pancreatic RNase digest correspond to four unique sequences in MS2 RNA (ref. 18), in positions 3,437–3,444 (GAAA-GAGC), 3,453–3,458 (GAAAGC), 3,473–3,479 (GAAA-GGU) and 3,496–3,501 (AGGGAC). This suggests that at least part of the binding site is located in the sequence 3,437–3,501 in the 3'-terminal untranslated region. The remainder of the pancreatic RNase fragments have no unique sequences and therefore cannot be located unam-

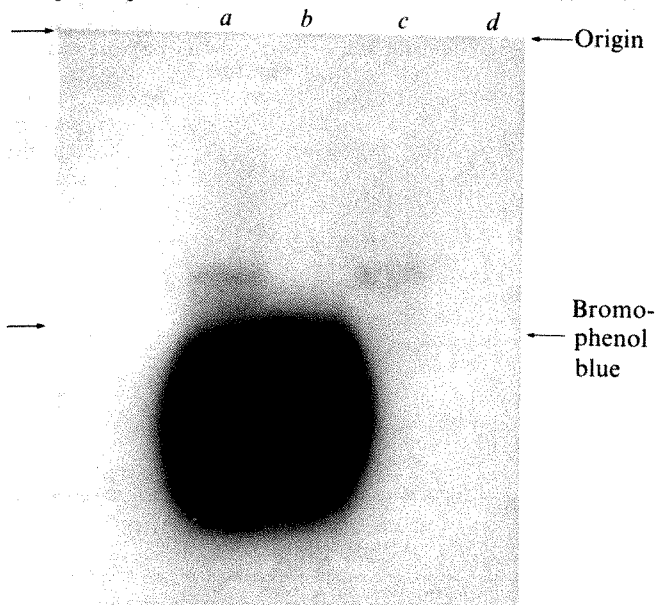


Fig. 1 Electrophoretic analysis of ³²P-MS2 RNA fragment remaining bound to IF3 after digestion with pancreatic RNase. ³²P-MS2 RNA (22.4 µg, prepared as described by Brownlee¹³) was incubated with IF3 (45 µg, prepared according to Lee-Huang *et al.*¹¹ to Step 4, 90% pure) in a total volume of 0.25 ml containing Tris-HCl, pH 7.8 (50 mM), NH₄Cl (50 mM), Mg(OAc)₂ (1 mM), GTP (1 mM) and β-mercaptoethanol (14 mM) for 12 min at 37°C. Pancreatic RNase (0.5 µg) was added and the incubation continued for a further 30 min at 37°C. A control incubation was carried out in the same way, but omitting the IF3. The incubations were worked up in one of two ways: (1) Direct phenol extractions (*a* and *b* (control)) of the reaction mixture and precipitation with ethanol. The precipitate was collected by centrifugation (30 min at 30,000 r.p.m.) and dried *in vacuo*. (2) Filtration through nitrocellulose (*c* and *d* (control)). The reaction mixture was cooled to 0°C and filtered slowly through nitrocellulose (Millipore, HAWP 02500, 0.45 µm). The filter was washed three times with 10-ml aliquots of buffer (Tris HCl, pH 7.8 (50 mM), NH₄Cl (50 mM) and Mg(OAc)₂ (1 mM)). RNA was extracted from the filter by shaking for 2 min at 70°C with 2 ml of 1:1 mixture of phenol and a buffer containing Tris-HCl, pH 7.8 (10 mM). The extract was cooled rapidly and the RNA precipitated with ethanol from the aqueous phase. A vertical slab gel (20 × 20 × 0.4 cm) containing 15% acrylamide and 0.375% bisacrylamide in a buffer containing Tris-HOAc, pH 8.2 (40 mM) and EDTA (1 mM) was prepared. The RNA samples were dissolved in 50 µl of this buffer containing 10% sucrose and bromophenol blue, applied to the gel and electrophoresed at 150 V for 16 h at 4°C. An autoradiograph of the gel was prepared. To prepare large quantities of the RNA fragment, the method described for slot *a* was used. In a typical experiment, 2 mg of ³²P-MS2 RNA was incubated with IF3 under conditions of maximal binding as determined by nitrocellulose filtration.

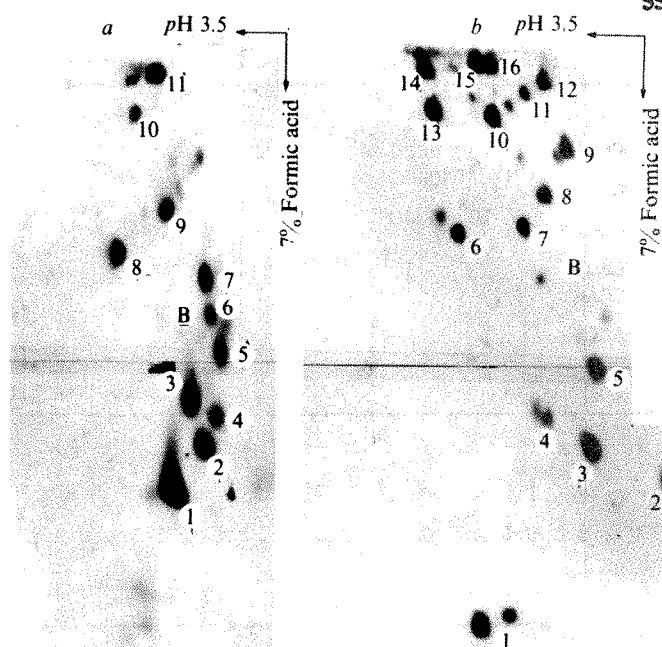


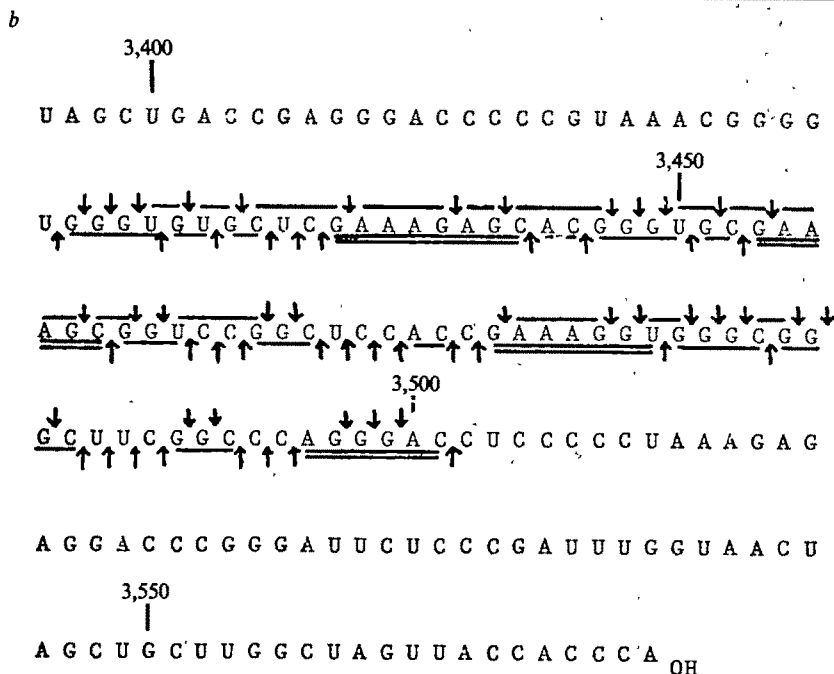
Fig. 2 Autoradiographs of fingerprints of *a*, RNase T1 and *b*, pancreatic RNase digests of the protected RNA. B indicates the position of the blue marker. The RNA fragment was prepared as described in Fig. 1, slot *a*. The band was eluted electrophoretically from the gel¹⁵ and fingerprints of RNase T1 and pancreatic RNase digests prepared¹⁶. The IF3 sample used in this preparation was 80% pure, as determined by SDS gel electrophoresis¹⁷. Fingerprints of RNase T1 and pancreatic RNase digests of the protected RNA prepared in exactly the same way, but using a homogeneous IF3 preparation differed only in the intensities of a few of the spots.

biguously. All except three (AGC, (AG,G)C and (AG,-AG,G)U), do however fit the sequence around the unique nucleotides mentioned. Of the RNase T1 digestion products, none is unique, but all except CCG, CCAG, AACG, UCUCG and (U₃C₃, AC, AU) correspond to the nucleotide sequence in the same region. The RNase T1 product U₃C₃-AC, AU) could not be unambiguously identified. It gives one spot on some fingerprints and two on others, but since it lacks a 3'-terminal G residue, it must represent the 3'-terminus of the protected region, which may account for its variability. The 3'-terminal untranslated sequence of MS2 RNA and the location of the digestion products within this region are shown in Fig. 3b. Thus, were the sequence continuous, its minimum length as determined by unique pancreatic RNase products would be 65 nucleotides, that is, twice the length determined by gel electrophoresis.

The pyrimidine-rich sequences 3,466–3,472 and 3,488–3,495 cannot be accounted for in the fingerprints. These regions may be less well protected against pancreatic RNase digestion and/or may suffer some breakdown during isolation of the fragment. But considering the small size of IF3, it seems more probable that these excisions indicate a binding site where different stretches of the RNA are held together in a specific secondary and tertiary structure. This may also account for the presence of the two pancreatic RNase and four RNase T1 products which originate from a more distant part of the MS2 RNA sequence. In this respect, the binding may be similar to the RNA-protein interactions observed between ribosomal RNA and ribosomal proteins where binding does not occur between continuous sequences in either of the participating molecules¹⁸.

So far as is known, the 3'-terminal untranslated region of mRNA has no role in the initiation process and the functional aspects of the binding of IF3 to this part of MS2 RNA remain open to speculation. It is interesting in this respect that S1 protein, which is supposed to participate in the same step as IF3 at the initiation of translation, binds near the 3'-terminus of QB RNA (ref. 10). IF3 may have

RNase T ₁ fragments		Pancreatic RNase fragments	
1. G	8. UG	1. U	9. (G,AAAG)C
2. CG	9. UCCG	2. AC	10. GGGC
3. AG	10. CUCG	3. GC	11. (G,G,AG)AC
4. CCG	11. (U ₃ ,C ₃ ,AC,AU,)	4. AU	12. (G,AG,AAAG)C
5. CACG		5. AGC	13. GGU
6. CCAG		6. GU	14. GGGU
7. AACG		7. GGC	15. (G,AG,AG)U
		8. (AG,G)C	16. (G,G,AAAG)U



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Fig. 3 *a*, Main oligonucleotides identified in the fingerprints shown in Fig. 2. Secondary analyses were carried out using standard techniques¹⁶. *b*, The 3'-terminal untranslated sequence of MS2 RNA (ref. 18) and the location of the RNase T₁ and pancreatic RNase digestion products. The RNase T₁ products are shown by a line above the sequence and the pancreatic RNase products by a line below. The four unique pancreatic RNase products are indicated by a double underlining. Arrows indicate nuclease cleavage sites.

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A histone-like protein from adenovirus chromatin

THE DNA in adenovirus is complexed with two viral specific polypeptides, VII and V, to form the compact core structure. There are 1,070 copies of VII (18,000 molecular weight) and 180 copies of V (45,000 MW) per viral genome of 36,000 base pairs¹. Corden *et al.*² have suggested that the combination of six copies of VII, one copy of V and 200 DNA base pairs form the basic subunit structure of adenovirus chromatin. Such arrangement is also characteristic of the eukaryotic chromatin subunit which is composed of eight histones (two each of H2a, H2b, H3 and H4) and a unit DNA length of approximately 200 base pairs (see ref. 3 for review). The major core protein, VII is highly basic; almost 23 mole per cent of the residues are arginine^{4–6}. A simple calculation reveals that the mass ratio of VII : DNA is 1 : 1 which is similar to the histone : DNA ratio in eukaryotic chromatin. This suggests that polypeptide VII–DNA interaction may be similar to that

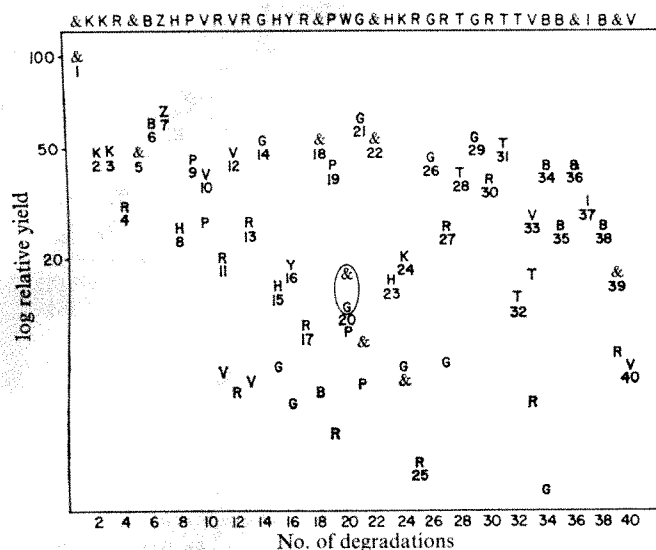


Fig. 1 Quantitative sequencing at the N terminus of VII. The log of relative yield is plotted against the step number. Automatic Edman degradation was done in an Edman-Begg sequenator¹⁸ (Illitron). The programme for phenylisothiocyanate coupling, cyclisation and cleavage has been described¹⁹. In our first sequence attempt of VII, step 10 showed a 58% carryover of proline. In the second run, the programme was modified by repeating the coupling and cleavage reactions at step 9. This minor modification in the programme reduced the proline carryover to less than 20% at step 10. Quantitative identification at each step was performed by H1 hydrolysis of the thiazolinones and analysis of the parent amino acids on a Durrum D-500 analyser. The data was plotted according to Smithies *et al.*¹⁹ and corrected for background and carryover. &, Ala or Ser.

of histones and DNA. Amino acid sequence studies have revealed an asymmetric distribution of basic residues in the histones. In particular, the amino end is highly basic and is implicated in DNA binding; the carboxyl end is less basic yet more hydrophobic and is involved in histone-histone interactions^{7,8}. To determine if the amino end of VII is also basic, we isolated polypeptide VII from adenovirus type 2 in milligram yield⁹ and sequenced its amino end by automatic Edman degradation (Fig. 1). We present here a partial sequence determination.

Table I gives the composite sequence data of the N-terminal 40 residues from polypeptide VII. This data is confirmed by two independent quantitative sequence studies (Fig. 1). Our actual sequence data extends to residue 48; however, residues 41-48 have only been sequenced once and are therefore not reported here. It is apparent that the

first 27 residues are enriched in basic amino acids (12–27 are basic). This is in contrast to the adjacent non-basic region which extends from positions 28 to 48, in which there are only two arginine residues at positions 30 and 45.

Figure 2 shows a diagrammatic representation of the charged amino acids in the amino third of polypeptide VII. The asymmetric basic versus non-basic 'domains' as described above are clearly illustrated. In addition, two clusters of basic residues, a triplet of Lys 3-Lys-Arg 5 followed by a longer cluster of His 23-Lys-Arg-Gly-Arg 27 are observed. In between the clusters, His and Arg are segregated at near regular intervals; Arg 11, Arg 13, His 15, and Arg 17 are equidistant from each other while His 8 and Arg 11 are longer by one residue. These non-random distributions of basic amino acids have also been observed in histones^{8,9}, especially in H4, an arginine-rich histone. In H4 (1) about 45% of the first 20 residues are basic, (2) there is a cluster of five consecutive basic residues Lys 16-Arg-His-Arg-Lys 20, and (3) basic residues 3, 5, 8, 12 and 16, are almost equidistant from each other.

The similarity of cationic residue distributions in VII and a highly 'sequence-conserved' histone, H4, immediately raises the possibility that the basic region in VII may also serve as a DNA combining site. The fact that functionally related yet distinctive modifications do occur at the amino end of both DNA-combining basic proteins tends to support this view. As shown in Fig. 2, Ser 1 and Lys 5, 8, 12 and 16 of histone H4 may be modified by phosphorylation¹⁰ and acetylation¹¹. Sung and Dixon¹⁰ have suggested that phosphorylation as well as acetylation of these residues reduces the strong electrostatic binding to DNA at this region of the molecule. This may in turn facilitate the 'proper binding'¹² and assembly of histones into chromatin subunits¹³. It is known that polypeptide VII is synthesised as a precursor molecule P-VII (Pro VII⁹) which is packaged in immature virions^{14,15}. At the final stage of virus maturation, a specific endoprotease¹⁷ processes a 20-residue fragment from the amino end of the molecule⁹ to give rise to polypeptide VII. It is likely that this processing event may be related to adenovirus chromatin assembly. We have shown⁹ that this precursor fragment is hydrophobic and may also be acidic (amide status unknown). Thus, the presence of this precursor sequence in Pro-VII may serve to modulate the strong electrostatic binding of the basic 'domain' to DNA. The net result is analogous to that exerted by enzymic phosphorylation and acetylation of histone H4. Subsequent processing event of polypeptide VII enables this region to regain its binding capacity and, thus, allows assembly of adenovirus chromatin.

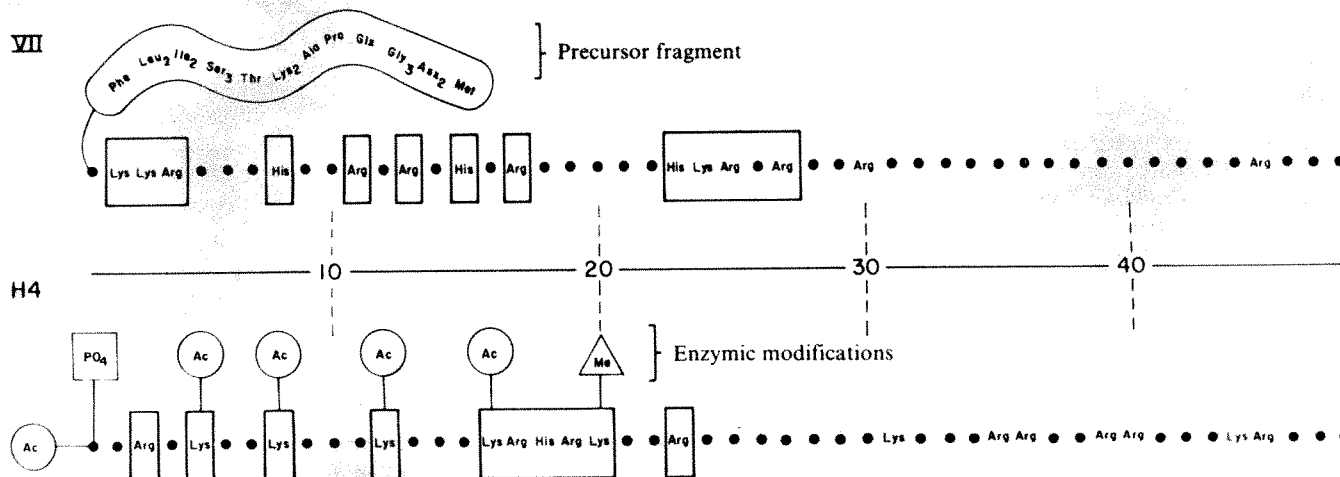


Fig. 2 Diagrammatic representation of the basic amino acid distribution at the NH₂ termini of adenovirus polypeptide VII and the arginine-rich histone H4. The 'basic domain' in the two proteins is illustrated. In histone H4 the possible sites of enzymatic phosphorylation (PO₄), acetylation (Ac) and methylation (Me) are shown. In polypeptide VII, a 20-residue precursor fragment exists at the amino end of the molecule before the final stages of viral assembly. Modifications seen in both systems may have similar roles in modulating the binding of the respective 'basic domain' to DNA.

Table 1 Summary of sequence data obtained from the N terminus of VII

Residue no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
HI	&	K	K	R	&	B	Z	H	P	V	R	V	R	G	H	Y	R	&	P	ξ
TLC	A	K	K	+	—	D	E	+	P	V	+	V	+	G	+	Y	+	A	P	W
Sequence	A	K	K	R	S	D	E	H	P	V	R	V	R	G	H	Y	R	A	P	W

Residue no.	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
HI	G	&	H	K	R	G	R	X	G	R	X	X	V	B	B	&	I	B	&	V
TLC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sequence	G	&	H	K	R	G	R	T	G	R	T	T	V	B	B	&	I	B	&	V

The thiazolinones were converted to the phenylthiohydantions and the PTH-amino acids identified by thin layer chromatography²⁰ (TLC). The sequence deduced is given in the last line of the table. &, Ala or Ser; ξ, gly + ala; X, α-aminobutyric acid; —, no PTH-amino acid present; +, not attempted.

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A new theory of olfaction based on dispersion-induced optical activity

OPTICAL activity is physically induced in achiral solutes dissolved in chiral solvents and is measurable as dispersion-induced circular dichroism (DICD)^{1–3}. An intermolecular vibrational coupling mechanism of the induction⁴ has been analysed theoretically⁵ and is here proposed as the primary physical process in olfaction. This new theory of olfaction extends the well-known qualitative vibrational theory^{6–7}, to encompass both chiral^{8,9} and achiral odorous substances^{10,11}. A quantitative aspect of the new theory is supported by a strong correlation of the solvent-induced rotatory power of achiral ketones with their effectiveness as alarm pheromones for the ant species *Iridomyrmex pruinosus*¹¹.

The rotatory inducibilities⁴ of 26 aliphatic ketones were measured by circular dichroism and isotropic absorption spectroscopy in their $n-\pi^*$ transitions as the chiroptical anisotropy factors¹² $g = \Delta\epsilon/\epsilon$, where $\Delta\epsilon$ and ϵ are the dichroic and isotropic molar extinction coefficients respectively. The values of g obtained for solution in (–)-bornyl acetate (BAC) and in (S)-propylene carbonate (PC) (refs 2, 3) are shown in Fig. 1 together with the pheromone activities of the ketones reported by Blum *et al.*¹¹ from laboratory and field trials.

The g values for acetone (point 1) and the homologous normal methylalkyl ketones from C₅ to C₁₂ (3–10) gave line a by the method of averages, line b was similarly obtained for the 3-, and 4-alkanones lacking α -substituents (11, 13, 14, 16, 18), and line c from the point of intersection of a and b and the experimental points for the cyclic ketones (24–26). The bioassay values for the ketones presented a target or star pattern centred on the common point of intersection of a , b , and c at acetone (1) with the natural pheromone, 2-heptanone¹¹, (5), occurring near the centre of the pattern and with pheromone activity decreasing radially. The near coincidence of these two independent, symmetrical, data patterns is compelling evidence of a correlation between olfaction and induced optical activity.

The linear relationships of the g values are most readily explained in terms of helical molecular conformations imposed by vibrational coupling¹³ on the achiral ketones by the chiral solvent and olfactory receptor molecules and will be discussed elsewhere. Such helical structures are, of course, inherent in naturally chiral compounds.

It is evident that DICD and infrared spectrophotometry should provide a quantitative *in vitro* method for evaluating olfactory response to odorous substances and mimics of natural chemical signal compounds.

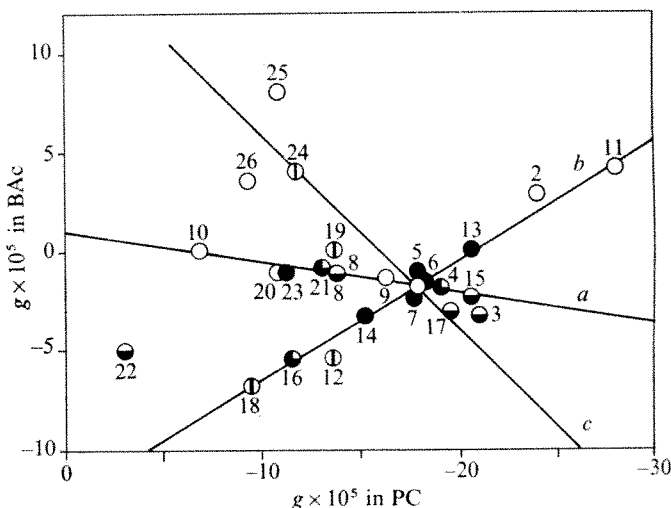


Fig. 1 Correlation of solvent-induced chiroptical anisotropy, g , of achiral ketones in (S)-propylene carbonate (PC) and (–)-bornyl acetate (BAC) with their bioassays as alarm pheromones of the ant *Iridomyrmex pruinosus* (Roger) taken from ref. 11. Pheromone activity increases with filling of the circles from empty at 0 to full at 4. A filled circle also designates the natural pheromone (5). $g = \Delta\epsilon/\epsilon$ was determined from CD and ultraviolet spectra run at $25.0 \pm 0.1^\circ\text{C}$ as described in refs 2 and 3. The ketones are: (1) to (10), the normal methylalkyl ketones from C₃ to C₁₂; (11) 3-pentanone; (12) 4-methyl-2-pentanone; (13) 3-heptanone; (14) 4-heptanone; (15) 5-methyl-2-hexanone; (16) 2-methyl-4-heptanone; (17) 5-nonanone; (18) 2,6-dimethyl-4-heptanone; (19) 3-methyl-2-butanone; (20) 3,3-dimethyl-2-butanone; (21) 5-hexen-2-one; (22) 2,4-dimethyl-3-pentanone; (23) 6-methyl-5-hepten-2-one; (24) cyclopropylmethyl ketone; (25) cyclodecanone; and (26) cyclododecanone.

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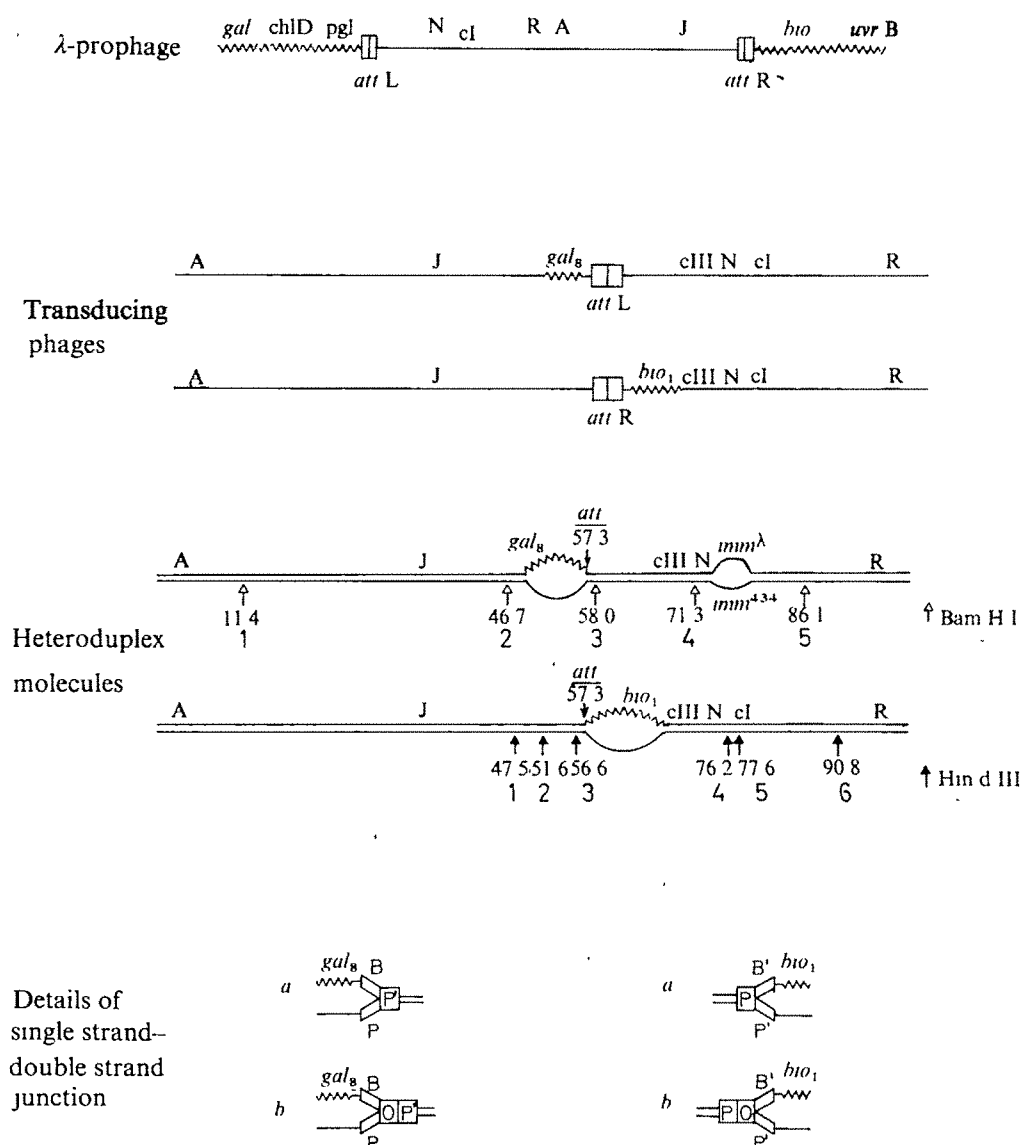
Precise location of the crossover region in the λ attachment sequence

A PREREQUISITE for studying the DNA-protein interactions involved in integration and excision of λ is the determination of the DNA sequences of the attachment sites. Useful sequence data can only be obtained if the position of the attachment site is known precisely. To focus on this site

we have used genetically- and physically-defined deletions and substitutions ending in *att* as reference points whose position relative to the sites of action of restriction endonucleases can be determined. In this way we have located the middle of the attachment site to within ± 10 base pairs in a molecule 46,500 base pairs long¹.

The specialised transducing phages λ bio and λ gal carry the right and left prophage end attachment sites respectively, with an adjacent substitution of bacterial DNA and a deletion of part of the opposite end of the prophage (Fig. 1). On hybridisation with normal λ molecules, the heteroduplex molecules formed have a single-stranded region, one end of which corresponds to the junction of bacterial DNA and the attachment site (Fig. 1). One end of one of the double-stranded pieces of DNA that remain after complete digestion of the single-stranded region with the single-strand specific S1 nuclease^{2,3} thus corresponds to this junction. All the available evidence points to this junction being within the attachment sequence and not at the end of it⁸⁻¹³. Thus the region of non-homology in the heteroduplex molecules of λ gal and λ includes the recognition sequences P and B (refs 9, 10), and the region of non-homology in λ bio/ λ heteroduplex molecules includes P' and B' (Fig. 1).

We determined the distance from Bam HI site 3 (ref. 5) to the crossover region by hybridising¹⁴ λ imm⁴³⁴ and λ gal₈ DNA and digesting sequentially with S1 nuclease and Bam HI. In each of five experiments two small DNA pieces were



detected, one 1,000 base pairs long (probably Bam HI cut 4 to *imm*⁴³⁴, 2.3% λ , 1,070 base pairs), and one 260 base pairs long (Fig. 2a). As a standard for comparison, we used ϕ X174 RFI DNA Hha I fragments, the lengths of which are known from DNA sequencing work¹³. Digestion of λ *imm*⁴³⁴ or λ *gal*₈ DNA with Bam HI and S1 singly or in combination and digestion of the DNA in the hybridisation mix with either enzyme alone does not give rise to any DNA pieces in this size range. The smaller of the two

pieces can be further positively identified as containing DNA between Bam HI site 3 and *att*, since it can be further digested with Hinf to yield the fragment δ (see below), and a fragment that is 215–220 base pairs long (Fig. 2c). Digestion of λ or λ *pbio*₁ DNA with Hind III yields two DNA fragments in the size range 10–1,000 base pairs. One is 620 base pairs long (1.3% λ) and probably represents DNA between Hind III cuts 4 (76.4% λ) and 5 (77.4% λ) (refs 6, 7). The other is only 130 base pairs long,

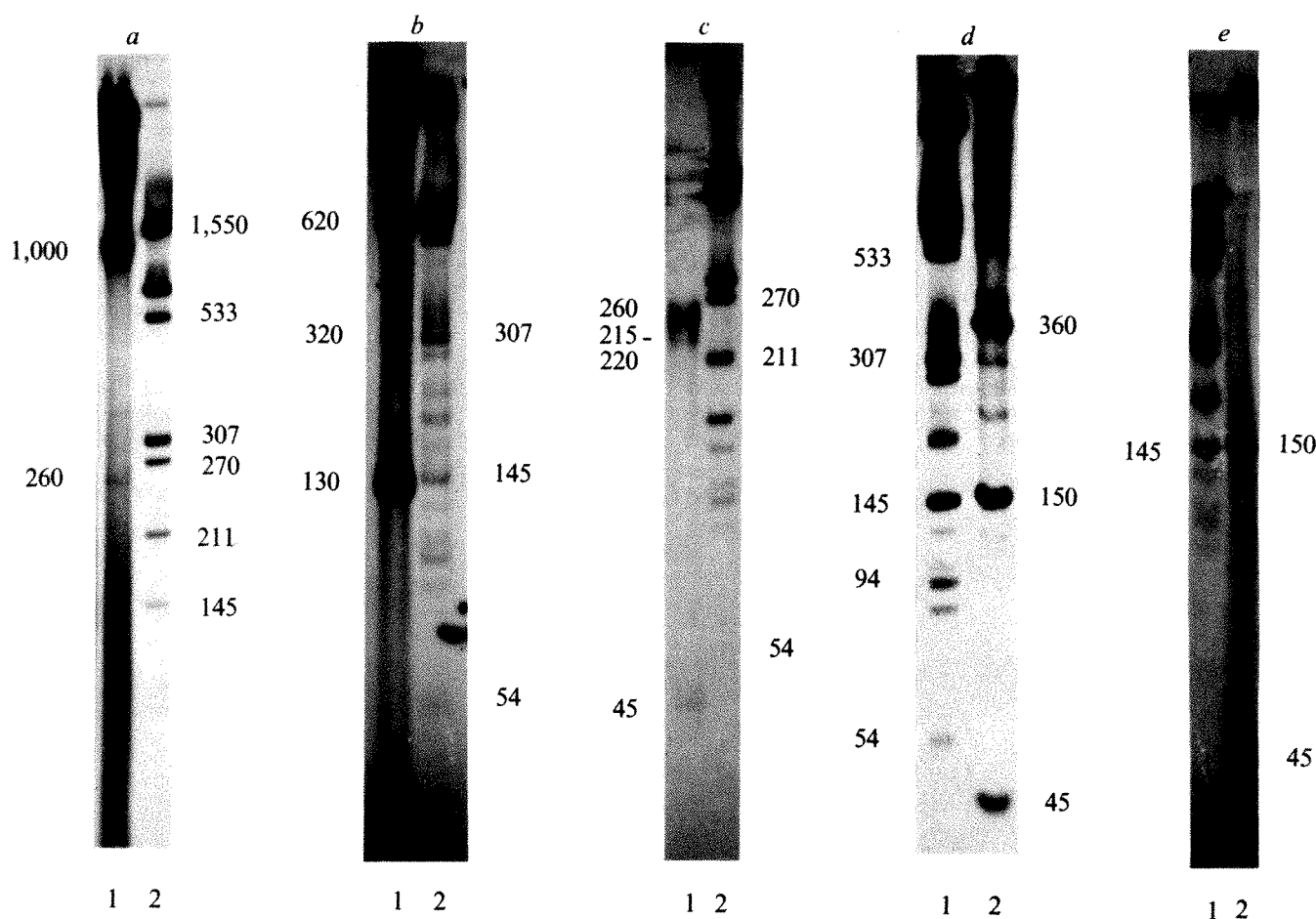


Fig. 2 Autoradiographs of DNA fragments in polyacrylamide gels. *a1*, Phage λ *pgal*₈/ λ *imm*⁴³⁴ heteroduplexes treated with S1 and Bam HI; *b1*, phage λ / λ *pbio*₁ heteroduplexes treated with S1 and Hind III; *c1*, fragment *att*-Bam HI cut 3 treated with Hinf; *d2*, fragment α treated with Hinf; *e2*, fragment α end-labelled with ³²P treated with Hha I. Hybridisation was carried out according to Fanning *et al.*¹⁴. Incubation with S1 nuclease was in 0.03 M Na acetate, pH 4.6, 10⁻⁴ M ZnSO₄, 0.1 M NaCl for 30 min at 22 °C. The DNA was then ethanol precipitated, washed with ethanol, dried and taken up in the buffer for the restriction enzyme digestion. Restriction endonuclease digestion was carried out at 37 °C in the following conditions: Bam HI (purified according to Wilson and Young¹⁹) in 6 mM Tris, pH 7.4, 6 mM MgCl₂, 6 mM 2-mercaptoethanol, 30 mM NaCl, Hind III (Miles Laboratories and Biolabs Inc.) in 6 mM Tris, pH 7.4, 6 mM MgCl₂, 30 mM NaCl, Hha I (gift of E. Winnacker, and Biolabs Inc.) in 6 mM Tris, pH 7.4, 6 mM MgCl₂, 6 mM 2-mercaptoethanol, 50 mM NaCl, Hinf (gift of A. Waltz) in 10 mM Tris, pH 7.4, 15 mM MgCl₂, 0.6 mM DTT. It was sometimes necessary to separate large fragments from small products of S1 digestion in a 0–20% sucrose gradient run at 41,000 r.p.m. in a Beckman SW41 ti rotor for 14 h at 4 °C before treating with the restriction endonuclease. The source of α (Hind III cut 3-Bam HI cut 3) was pMG 1409, a derivative of pSF 2124 (ref. 17) carrying the EcoRI C (ref. 18) fragment. The hybrid plasmid was constructed by *in vitro* ligation of the λ EcoRI fragment¹⁸ with RSF 2124 (ref. 17) linearised by EcoRI. The λ EcoRI C fragment had been purified using 0.8% Agarose gels and KI equilibrium gradient centrifugation²⁵. The ligation mixture contained 70 μ g of plasmid DNA and 1.5 μ g of λ EcoRI C fragment, in a total volume of 1 ml of 50 mM Tris, pH 7.5, 50 mM NaCl, 35 mM MgCl₂, 5 mM DTT, 0.066 mM ATP, with 0.5 U of T4 DNA ligase (Miles). Cells were transformed using the CaCl₂ technique²⁶ and transformants were selected on plates containing ampicillin (100 μ g ml⁻¹). Clones containing the hybrid plasmid were identified by their resistance to ampicillin and their failure to produce colicin EI. Plasmid DNA was purified according to Clewell and Helinski¹⁹ with minor modifications. Digestion of this plasmid DNA with Hind III and Bam HI gives α and fragments ~7,000 and 8,300 base pairs long. α can be prepurified on a 0–20% sucrose gradient run for 18 h at 41,000 r.p.m. at 4 °C in the Beckman SW41 ti rotor. DNA fragments were labelled *in vitro* by nick translation²⁰ in 50 mM Tris, pH 7.4, 10 mM MgCl₂, 0.1 μ M 2-mercaptoethanol, 5–10 μ Ci of one α -³²P-labelled nucleotide triphosphate (dried to remove ethanol if necessary), the three other deoxynucleotide triphosphates 0.05 mM, with 1–2 units DNA polymerase I (Boehringer, Grade I) for 1 h at room temperature. The reaction was stopped by heating at 70 °C for 10 min. Nick translation usually followed digestion with the restriction enzyme. Fragments were end-labelled by treating with bacterial alkaline phosphatase (Worthington BAPF) for 1 h at 37 °C, extracting once with phenol and once with ether, precipitating and washing with ethanol, taking the dried precipitate up in 20 μ l of a solution containing 1 μ M γ -³²P-ATP (NEN), 50 mM Tris, pH 7.4, 10 mM MgCl₂, 5 mM DTT and 1 mM spermidine, and incubating with 0.5–3 units of polynucleotide kinase (Miles Laboratories, Biolabs Inc.) for 30 min at 37 °C. Samples were subjected to electrophoresis on 8% polyacrylamide slab gels (14 $\times$ 30 $\times$ 0.2 cm). The running buffer was 50 mM Tris borate, pH 8.3, 1 mM EDTA. After running the gel at 15 °C at 200 V for 14 h, the gel was autoradiographed. The numbers adjacent to the bands indicate the numbers of base pairs of the respective fragments.

so there must be a hitherto undetected Hind III cut very close to one of the others. A new band appears when λ and λ bio_I DNAs are hybridised and digested sequentially with S1 and Hind III (Fig. 2b). In three separate experiments, the length of this DNA piece was determined as 320 base pairs. This band is not seen when the DNA in the hybridisation mix is digested with either enzyme separately, and represents DNA between Hind III cut 3 and the left end of the bio_I substitution, since the right end of the bio_I substitution is 9.7% λ (4,500 base pairs) away from the next Hind III cut^{1,6,7}.

The sum of the lengths of the Bam HI₃-att and Hind III₃-att DNA pieces is 580 base pairs. To obtain an independent estimate of this distance, we isolated the Hind III₃-Bam HI₃ piece (Fig. 2), which we call α . α can be cut by the restriction endonuclease Hinf to yield three pieces, β , γ and δ (Fig. 2d). If the 5' ends of α are labelled using polynucleotide kinase and then digested with Hinf, only γ and δ are labelled (Fig. 2e). A fragment identical in mobility to δ is obtained by Hinf digestion of the Bam HI-att fragment (Fig. 2c). Thus δ is to the right, γ to the left, and β in the middle. The lengths of β , γ and δ as determined by ten separate measurements of their mobilities in gels are 360, 150 and 45 base pairs respectively, giving 555 base pairs for α . No other fragments have been detected, and the radioactivity present in the three bands after nick translation with α -³²P-dGTP is in the ratio 7.4:3.5:1, which is similar to the ratio of the fragment lengths, 8:3.3:1. Endonuclease Hha I also cuts α twice, yielding three fragments that are 470, 65 and 15 base pairs long, their sum being 550 base pairs. Mbo_{II} also cuts α twice yielding three fragments 380, 90 and 75 base pairs long, their sum being 545 base pairs. These values agree very well with the value obtained from the sizes of the Hinf fragments. We therefore have four independent measurements of the distance between Hind III cut 3 and Bam HI cut 3; 550, 555 and 545 from the Hha I, Hinf and Mbo_{II} pieces, 580 from the Hind III-S1 plus Bam HI-S1 pieces. The difference is too large to be accounted for by errors of measurement. No significant change in the observed size of the Bam HI₃-att piece was seen on increasing the amount of S1 nuclease twofold or decreasing it fivefold, so digestion of the single-stranded region with S1 was complete. One possibility is that a fragment or fragments have a mobility that does not correspond well to its

real length²¹. A more interesting possibility arises from the fact that if a common region of homology^{22,23} is present in the attachment sequences of phage and host, the ends left by S1 in the attachment region will be at the junction between the common homology region and a recognition sequence (Fig. 1). In this case the ends should overlap by the length of the common homology region, so the discrepancy in the values obtained would indicate a common homology region of 20–25 base pairs (Fig. 3).

The position of the site of recombination is shown in Fig. 3. It is thus possible in this way to determine the physical map position of genetically-defined elements in large DNA molecules to within 20 nucleotides.

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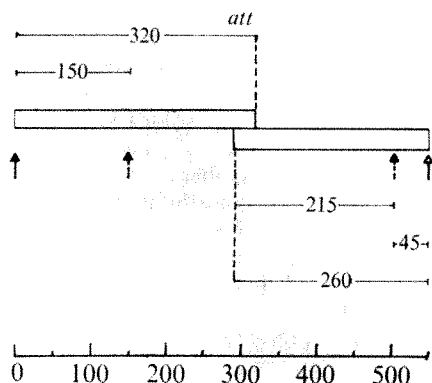
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Fig. 3 Map of the attachment site region of λ . The sizes of the Hinf fragments form the basis of the map. Digestion of the Hinf fragments individually with Hha I and Mbo_{II} places the Hha I cuts 15 and 80 base pairs from the Hind III cut, the Mbo_{II} cuts 90 base pairs from the Hind III cut and 75 base pairs from the Bam HI cut. Marini and Landy²⁴ have also found a 380 base-pairs long Mbo_{II} piece containing att. The restriction endonucleases Hpa I, Hpa II, Hae II, Hae III, Hind II, Hind III, Bam HI, Sac III and Pst I do not cut within this DNA segment. Filled arrow, solid tail: Hind III; unfilled arrow, Bam HI; filled arrow, dotted tail: Hinf. Lengths of the fragments are given in base pairs.



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Erratum

In the article 'Direct demonstration of β -globin mRNA in homozygous Ferrara β_0 -thalassaemia patients, *Nature*, **266**, 231–234, the author sequence should have read S. Ottolenghi, P. Comi, B. Giglioni, R. Williamson, C. Vullo, L. del Senno and F. Conconi. Figure 3 description line 2 should read Nuclear (a) and cytoplasmic (b) . . .

matters arising

Function of byssus threads in young postlarval *Mytilus*

STUDY of the settlement of postlarvae of *Mytilus edulis*, which live attached to filiform algae and hydroids, has shown that they migrate frequently^{1,2}. They detach themselves from their byssus, leave the substratum, are transported again by the water and re-attach on a new substratum. Like Sigurdsson *et al.*¹, we observed long mucous threads on young mussels and compared the drifting at the end of a long thread with the gossamer flight of young spiders, but believed that the aberrant primary byssus thread⁴ was involved chiefly in the settlement of the young. We tested this assumption. We placed a tuft of the alga *Polysiphonia* with many attached mussels in a transparent annular tank (diameter 1 m) in which a constant rotating current was generated. Four small racks with 1.5 m of nylon thread wound around them were placed perpendicular to the current. After 1 d some of the mussels, usually some hundreds, had resettled from the alga on to the threads.

Of the young mussels carried along by the current, occasionally one remained at the spot 2–10 cm downstream a nylon thread, apparently connected by the byssus thread, which had trailed behind and made contact with the substratum. At this anchored stage the elastic mucous thread protruded from between the valves at a point near the apex. Then the animal made swinging movements around this point over an angle of 60°, and in doing so pulled in the byssus thread, again behaving like a spider. Frequently a mussel was able to reach the substratum in this way, but the connection could also be lost. It seemed to be especially difficult for the mussel to grip the substratum with its long motile foot. Sometimes the substratum was abandoned again after short inspection. In such cases time was needed to perform a new anchoring procedure, and this corresponded with the time needed to secrete a new thread.

When nylon threads with diameters in the range 120–1,000 μm were stretched on racks in the sea, the thicker threads caught significantly larger mussels ($P < 0.001$), but such a difference consistently found in the tank was never significant. The average length of the young mussels caught on nylon threads varied in different experi-

ments between 900 and 1,500 μm , the smallest measuring 240 μm (metamorphosis), the largest 2,000 μm or more.

The behaviour of the migrating postlarvae may explain the high resettlement rates found at sea—600 specimens in 5 d—per metre of nylon thread stretched perpendicular to the direction of the current in midwater. In the tank a current of 20 cm s^{-1} was most effective. In the sea the water movement in waves is possibly more important for settlement than are tidal currents.

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Liquid immiscibility in $\text{K}_2\text{O}-\text{FeO}-\text{Al}_2\text{O}_3-\text{SiO}_2$

VISSE and Koster van Groos¹ present a new phase equilibrium diagram for the plane leucite-fayalite- SiO_2 in the quaternary system $\text{K}_2\text{O}-\text{FeO}-\text{Al}_2\text{O}_3-\text{SiO}_2$, in which the field of immiscibility differs significantly from that which I presented earlier², and from that presented by Greig on the binary $\text{FeO}-\text{SiO}_2$ (ref. 3) (Fig. 1). Here I suggest the reason for the discrepancy, as no discussion or explanation is given by Visser and Koster van Groos.

The few details presented on the laboratory procedures used in the new work¹ suffice to indicate that their results should differ somewhat from mine. First, the oxygen fugacity during their work was slightly higher, as they note, because they used molybdenum rather than iron containers. The resulting presence of more Fe^{3+} in the melts would almost certainly expand the field of immiscibility⁴. I am assuming that they were able to overcome the difficult problems of obtaining adequate transfer of oxygen through their evacuated system to achieve PO_2 buffering. Second, the use of a vacuum during the runs in their work might cause loss of

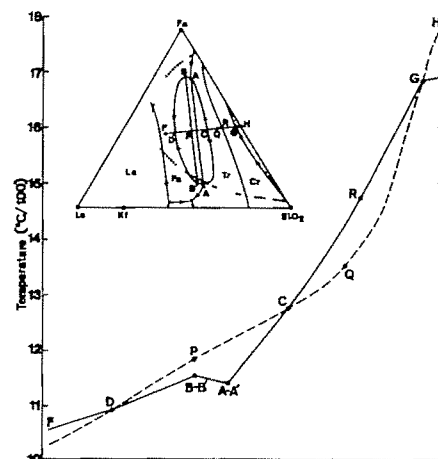


Fig. 1 Phase relations in the system $\text{K}_2\text{O}-\text{FeO}-\text{Al}_2\text{O}_3-\text{SiO}_2$ (inset), showing two fields of stable immiscibility (shaded), projected on the plane leucite (Lc)-fayalite (Fa)- SiO_2 , plotted in wt. %, from the early work of Roedder³. The outline of the larger field of immiscibility newly reported by Visser and Koster van Groos¹ is also shown (dotted). Tr, tridymite; Cr, cristobalite; Kf, K-feldspar. The T-X section along the line F-H is based on early work of Roedder³, plus the work of Watson¹¹ (point P) and Irvine¹⁰ (point Q). Two immiscible liquids exist in stable equilibrium along D-P-C and G-H.

potassium which would also increase the degree of oxidation of the charges. Third, the molybdenum containers must have put some molybdenum into the charges, either as Mo^{4+} or Mo^{6+} , both of which might be expected to increase the field of immiscibility⁴. I do not believe, however, that these three effects, even though additive, are adequate to explain the major differences found in the extent of the field of immiscibility.

Neither the work of Visser and Koster van Groos, nor my work gives the compositions studied nor the temperatures of the individual runs, but the numbers involved—~500 runs on 26 compositions in my work and >150 runs on an unspecified number of compositions in theirs—would both seem adequate to delineate the phase boundaries. (Although my diagram was presented as “preliminary” at the time, it has since been corroborated by additional data⁵.)

The differences between the two studies can be explained perfectly, however, by the assumption that some of immiscibility found by Visser and Koster van Groos took place during quenching, as the melt passed down through a field of metastable subliquidus immiscibility. The existence of such a field under the liquidus surface for silica in the system as I depicted it is self-evident from the geometry of the two immiscibility fields in a T-X section from the FeO-SiO₂ sideline through points C and D on Fig. 1. Furthermore, experimental evidence has been found for the existence of this metastable field, as well as for a metastable extension out under the very flat fayalite liquidus⁷. This evidence consisted of the presence of minute (~1 µm) globules having a high index, presumably high-FeO melt, in those glasses formed on relatively slow quenching, whereas the same charge quenched more rapidly yielded an optically uniform glass. Unfortunately, neither paper provides details on the quenching procedures. In my work, I quenched by dropping the iron foil envelope (~1×5×5 mm) containing the charge on to mercury while still in pure nitrogen. If the results suggested that phase changes had taken place on quench (for example, the minute globules, or dendritic crystals), faster quench was obtained by attaching platinum weights to the charge in the furnace to cause it to sink in the mercury on dropping⁸. All such minute globules were eliminated by this procedure.

The metastable sub-liquidus immiscibility indicated on Fig. 1 was originally drawn solely on the basis of the thermodynamic necessity of metastable extensions of the experimentally determined stable equilibria. It has been verified experimentally by Irvine¹⁰, who determined the stable and metastable phase relations for composition Q (Fig. 1) at 1,350 °C. The stable assemblage consisted of tridymite crystals and a liquid. Irvine was able to realise metastable immiscibility in this composition, with exsolved globules as much as 7 µm diameter, by superheating to 1,550 °C for 1–2 h, then supercooling for 10–15 min before quenching. The metastable solvus was 'roughly bracketed at 1,350 °C', which agrees well with the extension sketched in Fig. 1. Additional corroboration of the phase relations as sketched in Fig. 1 comes from some runs by Watson¹¹, establishing the 1,180 °C isotherm on the upper surface on the stable immiscibility field. He found this isotherm to intersect the stable two-liquid field at point P on the section (Fig. 1).

Metastable sub-liquidus immiscibility is commonly observed in the field of ceramics, and an extensive literature

exists⁹. Visser and Koster van Groos have used a very sensitive test for such immiscibility (electron scanning microscopy on etched surfaces), and they may even have detected such metastable immiscibility in samples that would seem optically homogeneous. Such metastability would explain many of the features they describe that are otherwise extremely puzzling. (Thus the field of immiscibility as drawn by Visser and Koster van Groos indicates a precipitous drop of 517 °C in the liquidus for silica in the system FeO-SiO₂ by the addition of only 1.35% FeO, and the metastable subliquidus extension of the immiscibility field would have to be even steeper). In view of the above evidence, I cannot accept their diagram as representing stable equilibria. I would suggest that the 'submicroscopic' immiscibility reported by them probably is metastable immiscibility from too slow quenching, and that only their coarser globules (as much as to 3 mm) represent stable immiscibility at furnace temperature.

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VISSER and Koster van Groos¹ have delineated a two-liquid phase field in the system K₂O-FeO-Al₂O₃-SiO₂ which is considerably larger than that reported by Roedder². Furthermore, the extent of the revised two-liquid region on the fayalite-silica join contradicts the phase equilibrium data for the system FeO-Fe₂O₃-SiO₂ (ref. 3). In our opinion the new data probably overestimate the extent of the stable liquid immiscibility field of this system for the following reasons.

(1) The submicroscopic glass-in-glass textures found in charges quenched from between 1,250 and 1,400 °C (ref. 1) are probably not equilibrium associations formed at the run temperature. They probably formed as the melt was quenched through a metastable immiscibility field below the liquidus surface. This explanation would account for the anomalous situation of certain conjugate liquid pairs forming

globules no larger than a few tenths of a micron, whereas macroscopic globules up to 3 mm in diameter form in charges differing only slightly in bulk composition¹. The textures figured¹ are identical in scale and morphology to those produced by quenching silicate melts through sub-liquidus immiscibility domes or by heat-treating supercooled silicate liquids^{4,5}.

(2) Previous studies⁶ show that minor amounts of molybdenum (up to 0.5 weight %) are dissolved by melts held in molybdenum capsules, where the oxygen fugacity is buffered at Fe-FeO by gas mixtures. The technique used by Visser and Koster van Groos¹ depends on the equilibration of melt and molybdenum sample container to buffer the oxygen fugacity of the charge, and is likely to be very favourable to the solution of Mo. Experiments in this laboratory show that the addition of 1 wt % molybdenum to the composition 40 fayalite, 26 leucite, 34 silica raises the upper limit of the two-liquid field by >30 °C. This exceeds the difference in temperature between the fayalite-tridymite cotectic and the leucite rich limit of the miscibility gap¹. Solution of molybdenum from the sample container⁶ would extend the stable two-liquid regions, as observed^{1–3}.

Using ⁸⁰Pt ²⁰Rh wire loop sample containers to minimise iron loss from the charge⁷ and controlled oxygen fugacity at the Fe-FeO buffer, we have confirmed the existence of a low temperature stable immiscibility field isolated from the fayalite-silica join, reported by Roedder². Glass beads formed using the wire loop technique have been analysed and show about a 10% loss of K₂O during an 18-h run; the effect of this loss on the geometry of the immiscible field is minor and where we have analysed the run products can be allowed for on the diagram. In conclusion, the statement that "at least for dry magmas an FeO-rich basalt must yield an immiscible granitic liquid during the later stage of crystallisation"¹ would seem to be premature.

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VISSER AND KOSTER VAN GROOS REPLY—The data which suggested to us that the liquid immiscibility field was significantly larger¹ than previously determined² were based on two independent phenomena. Runs at high temperatures (1,550 and 1,735 °C), made in molybdenum capsules in an induction furnace, showed extensive development of two immiscible liquids for compositions containing up to 7 wt % $\text{Al}_2\text{O}_3 + \text{K}_2\text{O}$. Spheres up to 1 mm in diameter were formed. The P_{O_2} was bracketed between the Mo–MoO₂ buffer and the Fe–FeO buffer (Fig. 1), and is estimated at 10^{-8} . Because the low-temperature two-liquid field at about 1,250 °C was found with compositions containing 8 wt % $\text{Al}_2\text{O}_3 + \text{K}_2\text{O}$, we thought the two immiscibility fields to be continuous. Therefore we investigated the intermediate temperature range between 1,250 and 1,500 °C. In the runs at these temperatures we found only submicroscopic separation between two liquid phases. We interpreted these as a stable phenomena because quench was relatively fast (5–10 s). Development of submicroscopic separation textures was identical to those at lower temperatures where we found a continuous range from large spheres (2 mm) to submicroscopic textures (Fig. 2a (1)). This suggested some kinetic problem for the phase separation at these temperatures. On the basis of the above we suggested a revision of the liquidus in the system K_2O –FeO– Al_2O_3 – SiO_2 .

Our recent findings, however, indicate that the major criticism of Freestone and Hamilton³ and Roedder⁴ is substantially correct. Thus, a large part of the immiscibility field as depicted in

our paper¹ is metastable, the stable part is in good agreement with Roedder's data². Before elaborating on this, we shall make some remarks on our experimental procedure. Most of our earlier runs were made by wrapping 250-mg samples in molybdenum foil, and sealing under vacuum in quartz tubing. We selected this technique because the wire loop method will most likely result in serious K_2O losses. Bow *et al.*⁷ report 20% loss (open capsules); O'Hara *et al.*⁵ 15% loss (open capsules); Freestone and Hamilton³ 10% (wire loop); while Naslund (wire loop)⁶ considers the loss insignificant. It is very important to keep the $\text{K}_2\text{O} : \text{Al}_2\text{O}_3$ ratio close to 1, or at least at a constant value, because change in this ratio effects the phase relations and the extent of the immiscibility field.

Several high temperature runs were made in an induction furnace, using molybdenum capsules sealed in platinum capsules. In our current research

we use sealed molybdenum capsules in an argon atmosphere. The gas flow through the furnace is set at the Mo–MoO₂ buffer. While these capsules develop hairline cracks during the run, K_2O losses are minimal. Furthermore, quenching times are reduced to about 0.25 s from the previous 5–10 s. As shown in Fig. 1, the prevailing molybdenum buffer, is Mo–MoO₂, the partial pressure of MoO₃ still very low.

In the experiments, the FeO loss to the sample holder was found to be insignificant; furthermore, the MoO₂ content of the silicate glasses is less than 0.1 wt % (detection limit) in all runs except those made in the induction furnace. Here the MoO₂ content of the glasses was about 0.6 wt %.

With our current method we repeated the high-temperature (1,735°, 1,550 °C) runs. We were unable to reproduce our previous results showing immiscibility textures. Comparison with the previous analytical data show that the $\text{K}_2\text{O} : \text{Al}_2\text{O}_3$ ratio of the earlier runs was very low (0.1) as compared with the new data (0.6–1.1). The MoO₂ content of these glasses is below the detection limit. It seems therefore that the low $\text{K}_2\text{O} : \text{Al}_2\text{O}_3$ ratio, possibly combined with the high MoO₂ content, resulted in the formation of immiscible textures. Results of the larger scale separation were in excellent agreement with our earlier runs. The submicroscopic separation features, however, were not reproduced in most runs owing to a better quenching method.

The submicroscopic phase separation was investigated with an energy dispersion in combination with a scanning electron microscope. Tie-lines of the co-existing liquids are much longer than those determined from the microscopic scale phase separation in the same sample. The lengths of these tie-lines roughly delineate the immiscibility field, as it was shown earlier¹, supporting the revised interpretation that this extend of this field represents the metastable extension of the two immiscibility fields found previously².

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Matters Arising

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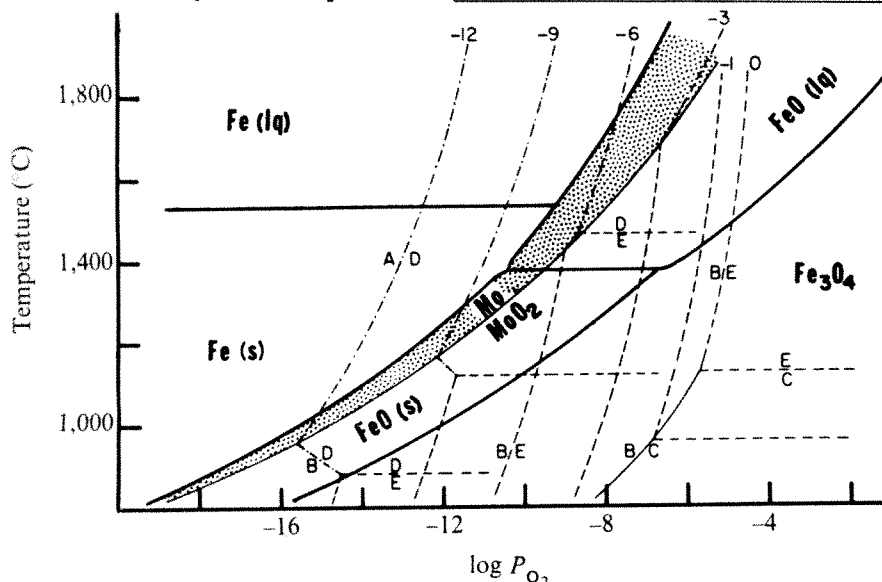


Fig. 1 Systems Fe–O and Mo–O. The heavy line depicts the stability relations of the Fe–O system. The lightly drawn lines depict reactions in the system Mo–O which are independent of the MoO₃ and (MoO₃)₂ vapour pressures, while the dashed lines depend on the (MoO₃)₂ pressure and the dot-dashed lines on the MoO₃ pressure. Runs were made in shaded areas. A, Mo (metal); B, MoO₂; C, MoO₃ (liquid); D, MoO₃ (vapour); E, (MoO₃)₂ (vapour). Data from ref. 7.

reviews

Popularising chemistry

A. R. Todd

New Worlds in Chemistry. By Martin Sherwood. Pp. 234 (Faber and Faber London, 1977.) £6.95

WITHOUT CHEMISTRY and the great industries based upon it, material civilisation as we know it has no future. The enormous changes which have taken place in the pattern of our daily lives during the past century or so have depended almost entirely on the progress of chemistry—the most central of the sciences whose tentacles reach out through all the others. Without it, the translation of most of the discoveries made in other sciences into practical, usable forms would simply not have occurred; and what is true of the past will be equally true in the future. Despite this, chemistry is undoubtedly less well known and well regarded than, for example, physics or biology by the general public, and it is rarely the subject of popular presentations on radio, television, or in the press. There is no single reason for this state of affairs, but from the many that could be adduced I would choose two as being of major importance.

The first is that the very ubiquity of chemistry and its products makes it a generally accepted part of everyday life to be noticed only when something untoward occurs, whereupon the public reacts with a display of frequently unfounded fear or panic. People, in other words, are aware of chemistry only dimly through its products or applications and are, for the most part, ignorant of or indifferent to the science itself.

The second is that chemistry, however absorbing it may be to its adherents, is inherently difficult to 'popularise'. To be understood, it requires acceptance of certain abstract concepts such as valency, bonding, and three-dimensional orientation of valencies, which have no real counterpart in everyday life. As a result the 'man-in-the-street' is usually unable to appreciate the chemist's ideas of molecular structure and interactions. This inability is not in my view simply due to chemical jargon. Be that as it may, popular expositions of the science have been few in number and successful ones even fewer. The appearance of *New Worlds in Chemistry* is therefore of more than passing interest.

In his new book, Dr Sherwood has done an excellent job of putting across a picture of modern chemistry in action. I found it extremely readable—journalistic here and there, perhaps, but probably none the worse for that. It seems to me that he has been successful because, accepting the ubiquity and maturity of the science, he devotes most

of his attention not to the search for spectacular events but to emphasising and exemplifying the part chemistry has played in a number of important developments in industry, in biology and in medicine, and in efforts to understand the origin of life on our planet.

The book opens with two chapters (47 pages in all) on the "Elements" and "Mechanics" of chemistry. Together they form a praiseworthy attempt to give a comprehensible yet reasonably accurate picture of the basis of chemistry today. I found it both readable and informative, but I was left wondering whether the general reader with no previous knowledge of chemistry would not still be in something of a fog after reading it.

The chapter on "Chemistry in the Beginning" gives a very—and perhaps unduly—full account of work on and theories relating to the development of organic compounds of varying complexity under pre-biotic conditions. Opinions vary about the value of much of the work on pre-biotic synthesis, since it is hardly surprising that organic compounds could be produced in conditions which would not support life as we know it. What would be far more

interesting would be the beginning of organisation—the appearance of coupled reactions under close control which is surely the real beginning of life; but of that we still know little.

The sections on the chemistry of life and death, on industrial chemistry and on the significance of chemistry in a variety of human activities from agriculture to fine art are well done, and the book closes with a chapter on "Chemistry and Tomorrow." In this chapter Dr Sherwood is brief and cautious in forecasting likely areas of advance but he devotes a good deal of space to the well worn question of the relationship between science and government, and the social responsibility of the scientist. These subjects are, of course, controversial but the author is right to point out the dangers inherent in the growing public awareness of the large scale effects which can be produced by modern technology and the need to prevent disillusion with science by the provision of adequate information. Taken as a whole *New Worlds in Chemistry* is a contribution to this end and it can be recommended to the general reader. □

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Vibrations of crystals

Vibration Spectra and Symmetry of Crystals. By H. Poulet and J. P. Mathieu. Translated by A. Simievic. Pp. xiv+571. (Gordon and Breach: New York, London and Paris, 1976.) £26.

PROFESSOR Mathieu is well known in France as an author of textbooks on the vibrational spectra of molecules and crystals. This book has evolved from one published in 1945. It is a translation of the original French edition of 1970; unfortunately, this is not stated anywhere in the book.

There are ten chapters on theory. They deal with the symmetry and internal structure of crystals, crystal dynamics, the symmetry of vibrations, and the classical and quantum theories of optical phenomena. The last chapter describes applications of the theory to infrared and Raman crystal spectra. There are seven appendices (on group theory and on X-ray and neutron diffraction), and a bibliography giving 185 references. The objective was "to give a systematic survey of the methods used to count the principal vibrations

of crystals, to determine their symmetry, to investigate their interactions with electromagnetic radiation and to establish the selection rules to which they are submitted".

The book has been written in a clear and helpful style, and will be useful to physicists and chemists concerned with the vibrations of crystals. Unfortunately, the translation is not always good—words such as "inequally", and phrases such as "integer multiple" and "allows to reduce their number" are to be found throughout; "et" occurs in the references instead of "and". An old value is used for the velocity of light, and SI units are not always used. There is a reference to Kitaigorodskii's 1961 book rather than to his *Molecular Crystals and Molecules*, published in 1973. There is no discussion of non-linear optical properties such as stimulated Raman scattering and the hyper-Raman effect.

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Linguistic theory

Integrated Theory of Linguistic Ability. By T. G. Bever, J. J. Katz and D. T. Langendoen. Pp. 432. (Harvester: Hassocks, Sussex, UK; Crowell: New York, 1977.) £14.95.

Is linguistics an empirical science like psychology or a theoretical science like logic? Is it, indeed, a science at all? One is bound to wonder about the status of a discipline that has as many schools and schisms as a political movement.

The great revolutionary figure is, of course, Noam Chomsky, the genius of transformational grammar. In 1965, he proposed a grammar, the so-called "standard theory", which formally specifies two different levels of syntactic structure. The surface structure of a sentence segments it into such constituents as noun phrases and verb phrases; its deep structure involves the same sorts of constituents but arranges them in a way that unequivocally identifies the subject and object of the sentence and other such grammatical relations; deep structure is mapped onto surface structure by transformational rules.

Chomsky's syntactic arguments for this grammar were compelling: most of the trouble has arisen over meaning, and the past decade has seen a continuing 'revolution within the revolution'. The standard theory took the deep structure of a sentence as a blueprint for its meaning, and the surface structure of the sentence as a blueprint for its phonological interpretation. But a group of radical linguists has argued that the theory is riddled with internal contradictions: grammar should derive the surface structure of a sentence not from some syntactic intermediary such as deep structure but directly from its meaning.

An alternative and 'revisionary' group, led by Chomsky himself, has progressively developed first the "extended standard theory", which allows both deep and surface structure to contribute to meaning, and then the "revised extended standard theory", which derives meaning solely from surface structure. There are still other alternatives, not to mention the numerous groups of linguists working outside the framework of transformational grammar.

The editors of this book—for it is a book of readings rather than the integrated text that its title page might suggest—are members of the 'revolutionary old guard' who wish to defend the standard theory, which they con-

sider to have been abandoned prematurely. To this end they have pulled together fourteen papers, most of which are by the editors and their collaborators, and most of which have appeared in print before.

The gist of their argument is that many of the phenomena that have seemed to motivate counter-revolutionary developments, in fact, defy linguistic explanation. Rather they are to be explained on the basis of the psychology of speech perception or pragmatic conventions governing conversation. The argument is remarkably persuasive, but its effect on the status of linguistics

is more questionable. Where many theories compete, it is natural to attempt to confront them with a wider set of empirical phenomena. By deliberately diminishing the set of relevant phenomena, the authors might seem to be moving linguistics towards the threshold of the comfortably insulated world of parlour politics.

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3-D imaging techniques

Three Dimensional Imaging Techniques. By T. Okoshi. Pp. 403. (Academic: New York and London, 1976.) \$15.00; £10.65.

INVENTORS have been fascinated by the problem of binocular or three dimensional (3-D) imaging for many years; G. della Porta (1540–1615) is reported to have drawn stereo pairs, but Wheatstone was probably the first to make a stereoscope. Since Wheatstone's time, many instruments and systems for producing 3-D images have been invented, several of them described in this book, but it must be admitted that none has yet proved to be the solution to become popular and make its backers multimillionaires.

In this book, there are chapters on vision (a brief treatment stressing depth perception), lens-sheet 3-D pictures (that is, pictures seen through lenticular screens, as in picture postcards and, about 25 years ago, in advertisements on the London underground), projection displays (the various devices for cine projection of 3-D pictures) and, of course, holography. Then there is a chapter on the information content of the different forms of 3-D imagery, and finally a chapter for unclassifiable systems; some of these are connected with computer-generated images and others, such as Gabor's cine projection screen in the form of a gigantic volume hologram, are unlikely to be realised in the foreseeable future. Notable omissions are the cine techniques popular in the early 1950s in which stereo pairs were projected on a single screen either in different colours, to be viewed through appropriately coloured spectacles, or in orthogonal polarisations, to be viewed through polarising spec-

tacles; there is also no mention of the Land Vectograph, a polarisation system used for producing prints or transparencies to be viewed through polarisers.

This book is at its best in the explanations of the technical details of the systems, and this is as it should be since the subject really depends on inventive developments. It is less impressive on the physical principles; for example, in the chapter on information a table gives the bandwidths required to transmit 3-D television by different methods, and figures of order 10^{12} Hz are given for wide-view holography; however, there are no comments on the prospects for achieving such a bandwidth and there is no indication of how the holographic image might be reconstructed. Much of the discussion in the chapter on information is aimed at television transmission but since this is probably many years ahead it is unlikely that the chapter will be very useful. Thus, the book is really an inventor's compendium or review on the subject and it is very good of its kind. Some of the practical details about lasers and holography are out-of-date, but this is because the book was originally published in 1972 (in Japanese). The present translation is very competently done by the author.

One may ask after reading Okoshi whether there will ever be a really good system for showing 3-D images? Perhaps not, although it is risky to prophesy; however, there is another method not mentioned by Okoshi, but known to and practised by the ancient Greeks, which gives quite good results—sculpture. Perhaps it will find a place among the Alternative Technologies.

W. T. Welford

W. T. Welford is Professor of Physics working in the Optics Section of the Blackett Laboratory, Imperial College, University of London, UK.

Hydrogen bond

The Hydrogen Bond: Recent Developments in Theory and Experiments. Vol. 1: Theory. Pp. viii+1-390. Vol. 2: Structure and Spectroscopy. Pp. viii+391-888. Vol. 3: Dynamics, Thermodynamics and Special Systems. Pp. viii+889-1,549. Edited by P. Schuster, G. Zundel, C. Sandorfy. (North Holland: Amsterdam, New York and Oxford, 1976.) Dfl. 450; \$173.75.

It has been a privilege and pleasure to review this treatise. The hydrogen bond is ubiquitous, and the growth in its studies phenomenal. When I recall my student days at Leicester, where Louis Hunter and his group made major contributions using classical methods to the study of the hydrogen bond—and at that time it was thought that this type of interaction occurred only with fluorine, oxygen, and nitrogen—we can realise the enormous strides which have been made in the experimental and theoretical approaches to this phenomenon. Matrix isolation techniques, neutron scattering, magnetic resonance spectroscopies, to name just a few, have added enormous depth and breadth to the subject.

We cannot readily avoid coming across hydrogen bonds in our work; whether we invoke them in proton abstraction mechanisms, or demonstrate them in structural studies. Only very recently it helped to rationalise an apparent anomaly we had observed: a precursor postulated in a room temperature reaction required, after isolation as a crystalline solid, an increase of 100 °C in temperature to give the same product. A crystal structure determination provided the answer (Cameron *et al.*, *Z. Naturforsch.*, **31b**, 1421, 1976): it showed the presence of a 12-membered ring hydrogen-bonded dimer, with some of the shortest recorded O-H . . . N, and N-H . . . S bonds.

These three volumes (detailed in the title) increase progressively in length and cover most aspects of the subject. Deliberately excluded are the mainly biological investigations, and also largely those devoted to the excited state and relaxation kinetics.

I personally found the studies of hydrogen-bonded systems by X-ray and neutron diffraction studies particularly fascinating. The two methods are critically compared, neutron scattering giving the longer X-H bond lengths. This technique also reveals that most hydrogen bonds are not strictly linear, and that in bifurcated hydrogen bonds,

the bifurcation is usually uneven.

The topology of the hydrated proton $H^+(H_2O)_n$ ($n=1, 2, 3, 4, 5$, and 6) is discussed. In most cases, at least two different structures could be considered for a given value of n .

Examples of symmetric $H_5O_2^+$ ions (for example, yttrium hydrogen oxalate trihydrate) and asymmetric $H_3O_2^+$ ions (for example, picrylsulphonic acid tetrahydrate) have been observed by diffraction methods. Similar studies by spectroscopic techniques show the important advances made in this field, and suggest that the symmetric diaquahydrogen ion $(H_2O.H.OH_2)^+$ is rather more prevalent than the asymmetric monoquaoxonium ion $(H_3O^+.OH_2)$.

Major advances in hydrogen-bonded systems have been made using nuclear magnetic resonance spectroscopy and the use of this technique is beautifully demonstrated for the liquid and the solid state.

Perhaps the last two chapters of this treatise, devoted to liquid water and ice respectively, demonstrate most vividly the diversity and complexity to which hydrogen bonding can give rise.

Although the distinguished contributors number almost forty and come from different nationalities, these volumes are written in excellent English, and overlap between chapters is

minimal. The editors must be congratulated. Most chapters cover the literature up to the period 1973-74, some with appendices and notes added in proof up to 1975, a very creditable achievement in an edited treatise. The volumes are beautifully produced and printing errors are scarce. It is a pity that blurring of pp 1,337-1,352, at least in my copy, slightly mars the overall impression. All volumes contain a subject index (covering all three). Volume 3 has also an author index.

Experimentalists (like myself) however, may well find volume 1 heavy going. It is a pity that individual volumes do not seem to be available.

I have so far only considered the quality of this treatise. Alas, we must also pay attention to its cost. A price of more than £100 will deter individual buyers and many libraries in this country. A crying shame as these volumes with their wide, almost universal, appeal ought to be on all library shelves.

I was instructed and stimulated by reading these volumes; I am saddened by the thought, that not enough scientists may be able to share this experience.

Robert A. Shaw

Robert A. Shaw is Professor of Chemistry at Birkbeck College, University of London, UK.

Immunological recipes

Techniques in Clinical Immunology. Edited by R. A. Thompson. Pp. 241. (Blackwell Scientific: Oxford, 1977.) Paperback £6.25.

BLACKWELL Scientific Publications have over the years accumulated a long list of 'titles' in immunology. The standard they and their authors have maintained is generally high. The books range from accounts of the proceedings of specialist conferences to the practical handbook under consideration here. This latter has eleven chapters on techniques commonly used in clinical immunology. It is parochial in that the kind of immunology under consideration is that practised in British hospitals and research institutes—it makes little or no attempt to be international.

Each chapter is written by a person who is involved on a day to day basis with the methods described. Every chapter starts with a short theoretical introduction to the topic before launching onto the collection of recipes which forms the bulk of the text. I am stuck, without doing the cooking myself, in trying to decide whether the

recipes work. They look alright.

The emphasis is toward the classical formulations of immunology rather than the new cell-mediated brand. There is, for example, no chapter on skin-testing. Little is said throughout the book of the indications for any particular test and only rarely about interpretation of results. Perhaps the most thoughtful chapter is that by Irene Batty on quality control. Few of the previous chapters on such disparate matters as the quantification of immune complexes, agglutination of cells or neutrophil leukocyte function tests (an intriguing choice this for an immunology text), give an indication of the way of handling the information gained. Dr Batty lays out in a clear way the basic necessity of quality control charts and describes the area of activity for which standardisation procedures are possible. Relatively few of the other chapters fall into this category.

It is a useful book carefully compiled which is too expensive for a paperback that might sell well.

A. J. S. Davies

A. J. S. Davies is Professor of Immunobiology in the University of London, working at the Chester Beatty Research Institute, London, UK.

Climatic reconstruction

Tree Rings and Climate. By H. C. Fritts. Pp. xii+567. (Academic: London and New York, 1977.) £16; \$35.

THIS is the first book to deal comprehensively with the relationships between tree-rings and climate, and their use to reconstruct past climates. By far the greatest volume of research in this field has been carried out in the United States and the majority stems from the Laboratory of Tree-Ring Research in Tucson, Arizona. Although tree-ring research has been carried out in the arid south-western United States and in Europe for over 50 years, it has been the development of a rigorous statistical approach, and the use of the newer multivariate techniques by Fritts and his colleagues at Tucson, that has transformed tree-ring studies from a dating technique to a climatic reconstruction science of world-wide application.

This book is thorough in its approach and assumes little previous knowledge. In particular, the author reviews the biology of tree growth from the molecular level through cellular to the whole tree and presents this account in a clear manner uncluttered with jargon. Some may feel that this aspect has been overplayed, as nearly 200 pages of the book are devoted to aspects of tree biology. Many scientists approaching dendroclimatology do so, however, from a non-biological background and would find the clear accounts in these early chapters most helpful. Following this biological introduction, the climate-growth relationships are examined and a series of models are proposed to explain a variety of observed relationships. There follows a rather brief account of the mechanics of tree-ring dating from sample collection, preparation and measurement to tests of reliability and the statistics used to describe tree-ring series. Included in this section is a mention of the techniques of time-series analysis that have been applied to tree-ring series.

The following chapters, which contain the real meat of the book, deal with the calibration of ring-width with climate and the concept of the response function. In this area, where the mathematical manipulations are difficult, Fritts succeeds in introducing each new concept and provides plenty of examples to show its application. In particular, he justifies and explains the use of eigenvectors of climatic variables in the multiple regression with ring-widths. He works through a number of examples and offers some

biological interpretations. This technique, which circumvents the problems caused by relationships between the variables, has so far been little used by other workers.

Although the response function of a single tree-ring series can be found and attempts made to interpret it in terms of growth models, it is seldom of any use for the reconstruction of past climate as the factors affecting growth are too complex. A factor such as high temperature may be found beneficial at some times of the year, and detrimental at others. Using several study sites, Fritts shows how multivariate techniques often allow valid reconstructions to be made.

The final chapters concentrate on examples of the more recent multivariate approaches—such as, reconstructions of lake levels, river flow, sea temperature, and even fish catches,

which are controlled by sea temperature. The last chapter gives in some detail the recent attempts by Fritts and his colleagues at pressure reconstructions using large numbers of tree-ring stations in the United States. This chapter also looks in detail at the various techniques of verification used to test the validity of the pressure reconstructions.

The book is well produced and clearly printed, and is remarkably free from minor errors. It should be of interest to all climatologists, and to a wide range of biologists, and its readable style compensates for its considerable length and detail. The explanations of multivariate techniques may well be of value to workers in entirely different fields.

Jon R. Pilcher

Jon R. Pilcher is Lecturer in Palaeoecology at Queen's University, Belfast, UK.

Chemical history

Chemists by Profession: The Origins of the Royal Institute of Chemistry. By Colin A. Russell with Noel G. Coley and Gerrylynn K. Roberts. Pp. x+342, 28 plates. (Open University Press, in association with The Royal Institute of Chemistry, 1977.) £9.50.

THE social history of science may seem a vague or obscure enterprise; but the attempt to discover what it felt like to be a chemist or physicist in the past cannot but be interesting, and is valuable because it casts light on that great majority of scientists who do not have a process, a genus or an equation named after them. This history of the Royal Institute of Chemistry, a body which is this year celebrating its centenary, is more than a benign tribute; it is a critical history, with particular emphasis on the gradual emergence of chemistry as a profession as well as a discipline. It is especially strong on the earliest years of the Institute (which did not become Royal until 1944), and indeed on the years before the Institute was founded—we do not get to the birth of the Institute until nearly half way through the book. This emphasis is completely justified by the authors, because unless one had understood what the position of chemists was before 1877 one would not understand the Institute and its problems, many of which have continued well beyond 1877.

The authors trace chemistry back not only to alchemists but also to assayers and pharmacists; and the tension between academics, chiefly preoccupied with the increase of knowledge but also anxious to promote careers for their graduates, and the

practical men, or practising chemists, has remained ever since. At the British Association, Section B devoted to chemistry dates back to 1835; while in 1841 both the Chemical Society—the first in the world, but many years after the Geological and Astronomical Societies—and the Pharmaceutical Society were founded. The Chemical Society was a learned society, run by academics; but with the rise of chemical industry, the invasion by science of industries like brewing, and the passing of laws against pollution, the industrial chemist and the consultant analyst appeared upon the scene in greater numbers, and demanded professional regulation.

On the one hand, there was pressure (especially from the academics) for high standards; and on the other, pressure to include and register all those working in the field of chemistry, many of whom could not pass examinations of honours degree standard. In the event, it was only in the last third of its century that the Institute took these wider responsibilities as its most pressing concern; in the earlier years its professional functions were narrower than they are now—when it has also merged with the learned society from which a hundred years ago it separated. This history does not answer all the questions that one might want to know; it is generous to previous historians, and also to participants in the history, and does not dilate overmuch upon money, patronage or feuds; but it provides a splendid account of its subject, which will interest not only historians but also scientists.

David Knight

David Knight is Senior Lecturer in History of Science at the University of Durham. He has recently published The Nature of Science (Deutsch, 1977).

obituary

A. J. Haagen-Smit

THE FIELD of air pollution lost one of its most vocal and colourful workers when Professor A. J. Haagen-Smit died on 17 March 1977 of cancer in Pasadena, California.

The objective facts are that Arie Jan Haagen-Smit was born in 1900 in Utrecht, Holland, and obtained his B.A., M.A., and Ph.D. from the University of that city, obtaining the last degree in 1928. After obtaining his doctorate, he was successively head assistant in organic chemistry and lecturer until 1936, when he went to the U.S. as a lecturer in biological chemistry at Harvard, spending the 1936-37 academic year there. In 1937 he joined the faculty at the California Institute of Technology as an associate professor, as a part of a conversion of that institution from a local trade school to its present level of excellence, begun under the late Dr Robert Millikan. Haagen-Smit retired at CalTech in 1971 as Professor of Biochemistry. He also served as executive officer of the department of biochemistry and director of the excellent plant environment laboratory there.

His early research interests were in natural products, particularly essential oils, although he made major contributions to the chemistry of plant alkaloids, plant hormones, and the chemistry of microorganisms. Towards the end of World War II, the Los Angeles area began to experience an increased incidence of haze, accompanied by eye irritation and damage to plants. In the late 1940s this had become a sufficiently frequent occurrence to generate public pressure for its abatement, and it was promptly termed "smog" by some supposed analogy to the more venerable phenomenon then characteristic of London. A sizeable effort was made, derived from this analogy, to control emissions of sulphur dioxide in the area. If anything, the situation got worse.

Concerning the events that followed, Haagen-Smit himself subsequently told several stories. I am personally inclined to credit the one he told me when I first met him, in the mid-1950s. Remember that he was particularly concerned with essential oils, and therefore had a very highly trained nose. He said that the current explanation (that it was all a matter of sulphur emissions) did not fit with what he

smelled on a smoggy day, which was much more reminiscent of oxygenated organic compounds. One day he was leafing through some back copies of *Chemical Abstracts*, and chanced upon a report of a Swiss patent from, if memory serves me correctly, the late 1930s. This patent claimed a process for producing mixed oxygenated compounds from hydrocarbons by photolysis in the presence of nitrogen dioxide. He immediately set up a benchtop experiment with some nitrogen dioxide and a bit of 3-methylheptane, and exposed it to a conveniently available ultraviolet light. After a time, he sniffed the resulting brew, and found that the odour closely resembled that of the outdoors air on the same smoggy day. I remember chuckling with him over the question of whether the holder of the Swiss patent should sue the City of Los Angeles for infringement.

Given these facts, it was obvious to Haagen-Smit that the source of Los Angeles pollution was primarily the automobile and secondarily the petroleum refineries that are interspersed throughout the city. (In fact, I have heard it theorised that the initial wartime problem stemmed from the injudicious selection of a site for a plant to manufacture butadiene, a key ingredient in synthetic rubber.) Needless to say, Haagen-Smit was violently attacked. He had shown that ozone was one of the products of the photochemical reaction. Some of the local industries heavily sponsored a local eccentric who purported to have data showing that the ozone was all of natural origin. Industry also funded local research institutions in the construction of rather elaborate laboratory facilities specifically for the purpose of disproving his theory. An elaborate superstructure, the Air Pollution Foundation, was set up to administer these early contracts. Techniques and instruments were devised or adapted for the measurement of a number of the key ingredients in the Los Angeles atmosphere, and these were produced commercially by local scientific instrument makers, particularly by the Beckman Instrument Company. It is probably no exaggeration to say that modern atmospheric chemistry was born from the researches of Haagen-Smit and of those hired by the affected industries to refute him. While there were a few details needing correction, the sole result of the studies was to

support Haagen-Smit's original theory.

Once the issue was settled by the acquisition of further knowledge, Haagen-Smit received numerous appointments, honours, and awards. He served for a time as chairman of the California Air Resources Board and of the U.S. President's Task Force on Air Pollution, as well as serving as a member of numerous other committees. He worked briefly as a consultant for Southern California Edison, and had a signal role in the development of the technique of reducing nitrogen oxide emissions from power plants by two-stage combustion. He was founding editor of the *International Journal of Air Pollution*, but found the work uncongenial and passed the job on to me at the end of the first year. Nevertheless, he served on the Editorial Advisory Board until last year, through two metamorphoses; the journal is now called *Atmospheric Environment*. Honours included membership in the National Academy of Science and receipt of the Los Angeles County Clean Air Award, the Chambers Award of the Air Pollution Control Association, the Hodgkins Medal of the Smithsonian Institution, the B. Y. Morrison Award of the U.S. Department of Agriculture, the Honor Scroll of the American Institute of Chemists, the Fritzsche Award and the Award for Pollution Control of the American Chemical Society, the Cottrell Award of the Research Corporation of America, the John and Alice Tyler Ecology Award of Pepperdine University, and the National Medal of Science. In his native country he was made Laureate of Labour by the Netherland Chemical Society, and he was made Knight of the Order of Orange Nassau. It is almost unnecessary to add that he belonged to a lengthy list of appropriate professional societies.

However, it is not for his honours that his many colleagues throughout the world will remember. "Haagy." Rather we will remember him for his warm humanity, his unflagging humour and his scientific insight. It would be a signal service to the science historians of the future if someone could take the time and the trouble to begin collecting anecdotes of the man. It is perhaps illustrative of his essential humility that I have found it necessary to consult a half dozen biographical sources to compile the above list of

honours; he rated them sufficiently unimportant that he seems to have given no one source the complete list.

A couple of years ago a joke made the rounds in which God's intent to create heaven and earth was completely thwarted by a demand from the Heavenly Environmental Protection Agency that he first file an environmental impact statement. As I fell asleep the night after learning of Haagy's death, that story suddenly returned to me with the thought, "He's got some help; now Creation can proceed!"

James P. Lodge

Donald Reid

DONALD REID, Professor of Epidemiology at the London School of Hygiene and Tropical Medicine, died suddenly of a heart attack on March 26 at the age of 62. His standing as an epidemiologist had few equals, in Britain or in the world.

He was born in a small Scottish fishing village, the son of a Post Office linesman. The family was poor and survived only through his mother's courage and prudent housekeeping. In later life he still preferred a bus to a taxi, and in research he would think long and hard about efficient study design, and how to salvage something if the primary aim were to be frustrated. If "plan A" failed, he always had "plan B" ready.

After qualifying in medicine he entered RAF Bomber Command in 1939, and there laid the foundations of his own career and of psychiatric epidemiology by analysing the relation between stress and neurotic illness. He showed that breakdown correlated highly with current casualty rates in a particular unit, but not at all with total flying hours. After the war he joined Bradford Hill at the London School of Hygiene and Tropical Medicine, and he succeeded him on his retirement in 1961. In a discipline where data are largely observational and often of poor quality he practised and taught scepticism and meticulous accuracy; but where others discounted the research potential of vital statistics, or surveys using crude clinical examination techniques, he showed that even inferior data can be valuable provided that their errors are recognised and quantified.

Most of his work was in relation to respiratory disease, later extending also to cardiovascular problems. Using national sickness-absence data for postal workers he showed the importance for bronchitis of exposure to air pollution: an excess in absence rates in areas with higher fog frequency was specific for bronchitis and for out-

door workers. By similar methods he identified the tuberculosis hazard for workers in bacteriology laboratories and mortuaries. He was a leading contributor to our recognition of the effects on health of cigarette smoking, demonstrating its interactions with air pollution in the causation of bronchitis, and with other coronary risk factors in the occurrence of heart attacks. He was particularly delighted by his recent election as an honorary fellow of the American College of Cardiology, and at the time of his own death he was working on the predictors of death from coronary heart disease.

His reputation stood as high overseas as at home. His international studies of migrants compared their disease rates with those of their native-born contemporaries in the host country and their siblings at home, thus permitting separation of genetic and environmental influences. He had friends in almost every country, and was much in demand as lecturer, chairman and consultant. Many may have thought that his talks and opinions were given impromptu, but in fact they were always meticulously prepared. Similarly his confident and optimistic manner of belied painful inner self-questioning and nervousness.

His colleagues and students will remember him for his humour (often directed against himself), his warmth and unlimited concern with their well-being, and his complete public and private integrity. He leaves his wife and two daughters; and countless friends in many countries.

Geoffrey Rose

M. S. Vallarta

DR MANUEL SANDOVAL VALLARTA died on April 18 in Mexico at the age of 78 after a long and distinguished career. He was particularly well known for his theoretical work on the geomagnetic effects on cosmic rays.

Together with the late Abbé Lemaitre, Vallarta solved to first order the problem of the cosmic ray intensity distribution in the geomagnetic field. This was done in two stages. First, the "allowed" and "forbidden" directions for cosmic ray particles in the geomagnetic dipole were determined, using the newly devised BUSH differential analyser at MIT, by generalising the earlier treatment of Stormer. Secondly, by an elegant application of Liouville's Theorem, he and Lemaitre showed that for an isotropic cosmic ray flux the intensity at the earth in the allowed direction is the full intensity prevailing outside the geomagnetic field whereas that in the forbidden directions is zero.

This work provided the basis of our understanding of a wide range of

cosmic ray geomagnetic effects and led directly to two particularly important results. First, in predicting an East-West asymmetry in the cosmic ray intensity at low geomagnetic latitudes whose sign depended on whether the primary cosmic rays were positively or negatively charged, it made possible the experimental proof that the primary particles are predominantly of positive charge. Secondly, the calculations of geomagnetic cut-off energies as a function of position in the geomagnetic dipole, which were carried out in great detail by Vallarta and his students, permitted for the first time a determination of the form of the cosmic ray energy spectrum in the geomagnetically sensitive region. Subsequently, the use of Liouville's Theorem, pioneered in this context by Lemaitre and Vallarta, has been extended beyond the field of cosmic rays to the general case of charged particle motion in magnetic fields of all kinds, whether they be laboratory accelerators, plasma containment machines or situations where particles are accelerated or trapped in the naturally occurring magnetic fields of a wide variety of astronomical objects.

During the course of his lifetime Vallarta contributed notably to the prestige and advancement of science and learning generally in Mexico. He occupied a number of important posts in education and the administration of science, both in that country and in the wider international scene. In particular, he was *inter alia* and at various times President of the Mexican National Institute of Scientific Investigation, Member of the National Commission for Nuclear Energy, President of the Latin American Council for Cosmic Ray Studies, Member of the International Commission of Weights and Measures, Sub-secretary for Public Education for Mexico City, President of the Institute for Mexican Cultural Relations in North America and Chairman of the Scientific Council of the International Centre for Theoretical Physics in Trieste. He was the recipient of many academic honours in Mexico, Latin America and elsewhere. These included membership of the Pontifical Academy of Sciences, the Colegio Nacional of Mexico, and the American Academy of Arts and Sciences. In 1952 he was appointed a Knight of the French Legion of Honour.

Manuel Vallarta was outstanding in his generation and for many years he has been a familiar figure to successive generations of cosmic ray researchers. His perceptive and penetrating questions will be missed at future meetings and for all who knew him personally his death represents the loss of a good and kindly friend. He is survived by his wife Maria Luisa.

H. Elliot

announcements

On the Move

Dr J. P. Caen, after a sabbatical year in the department of Pharmacology, University of Cambridge, UK, back to the Research Unit INSERUM 150, Hôpital Lariboisière, 2 rue Ambroise Paré, 75010 Paris, France on 1 July 1977.

Dr N. P. E. Langham, School of Biological Sciences, University Sains Malaysia, Penang, Malaysia, to School of Natural Resources, University of the South Pacific, Laucala Bay, Fiji, on 26 April 1977.

A. Albert, Research Professor in Department of Pharmacological Sciences at State University of New York, Stony Brook, New York 11790, to Emeritus Professor in the Research School of Chemistry, Australian National University, Canberra PO Box 4, ACT 2600, Australia.

Dr S. G. Jennings, formerly in the Department of Physics, Science Laboratories, University of Durham, moved to the Atmospheric Sciences Laboratory, White Sands Missile Range, New Mexico 88001, USA, in November 1976.

Details of changes of department, sabbaticals, where leave will be taken and so on should be sent to On the Move; there is no charge for this service.

Foreign members of the Royal Society elected on 21 April 1977

S. Ebashi, Tokyo University. **I. M. Gelfand**, Moscow University. **E. Hückel**, University of Marburg. **E. Katzir**, Weizmann Institute of Science, Israel.

New Information Services

The Natural Environmental Research Council has set up a Marine Information and Advisory Service (MIAS) which has developed a computerised storage and retrieval system for marine data. For further details contact The Publicity Officer, MIAS, Institute of Oceanographic Sciences, Wormley, Godalming, Surrey, UK.

The Scientific Documentation Centre Ltd has set up weekly Current Awareness Services, handling data on many energy topics. For further information contact Scientific Documentation Centre Ltd, Halbeath House, Dunfermline, Fife, UK.

Person to Person

Wanted, 3-bedroom furnished house or flat for visiting Professor of pathology and wife and 2 teenage children July 1 1977–June 15 1978. Contact G. L. Davis, The Jewish Hospital of St Louis, 216 South Kingshighway Boulevard, St Louis, Missouri 63110 USA.

Exchange 4-bedroom furnished house in Loenen aan de Vecht, 20 min Amsterdam, for 3-4-bedroom house 1 h north or east of New York City 15 September 1977–15 September 1978. Contact G. Mulder, Department of Pharmacology, Free University, Amsterdam, Netherlands.

Marine biologists, aquarists, mariners, naturalists, fishermen. Your observations solicited for scientific work. Author collecting unusual/interesting observations of fresh- and saltwater fish schools, especially observations of spawning. Please give as much detail as possible. Quotations from ships' logs, diaries best. Include location of sighting, date, time of day, kind of fish, behaviour details, observer's name. G. Lorton, Biology Department UVa. Gilmer Hall, Charlottesville, Virginia 22903, USA.

Exchange 4-bedroom detached house in Harpenden, Hertfordshire, 10 min station, 35 min St Pancras, for house in Perth, Western Australia July 1977 to July 1978. Contact G. G. Briggs, Rothamstead Experimental Station, Harpenden, Hertfordshire.

Canadian seeks family accommodation in or near London for one year from August 1977, or exchange house in Montreal. Contact L. Polak, French Department, Birkbeck College, Malet Street, London WC1, or F. McGilly, 4220 West Hill Ave, Montreal 4HB 2S7 PQ Canada.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

Meetings

13 June–2 July, **Techniques of Developmental Biology**, Rio Piedras, Puerto Rico (G. C. Candelas, Biology Department, University of Puerto Rico, Rio Piedras, Puerto Rico 00931).

20–22 June, **4th International Symposium on Mass Spectrometry in Biochemistry and Medicine**, Riva del Garda, Italy (The Italian Group for Mass Spectrometry in Biochemistry and Medicine, c/o Mario Negri Institute, Via Eritrea, 62-20157 Milan, Italy).

27 June–1 July, **Workshop on Coastal Pollution Control**, Athens, Greece (WHO, Regional Office for Europe, 8 Scherfigsvej, DK-2100 Copenhagen, Denmark).

28 June–1 July, **Working Group on Constraints in Mental Health Services**, Cork, Ireland (WHO, Regional Office for Europe, 8 Scherfigsvej, DK-2100 Copenhagen, Denmark).

29–30 June, **Strategy and Tactics of Control of Migrant Pests**, London (The Royal Society, 6 Carlton House Terrace, London, UK).

30 June, **Laser Processing of Engineering Materials**, Heathrow Airport (C. D. DesForges, Fulmer Research Institute Ltd, Hollybush Hill, Stoke Poges, Slough, Bucks, UK).

30 June–1 July, **The Evolution of Metalloenzymes, Metalloproteins, and Related Materials**, Brighton (N. Birch, Department of Biochemistry, 9 Hyde Terrace, Leeds, UK).

4–5 July, **Conference on Instrumentation for Ground Vibration and Earthquakes**, Keele (The Institution of Civil Engineers, Great George Street, London SW1).

6–7 July, **Profits, Projects and Solar Energy**, London, UK (J. P. Schlesinger, Hyams & Co., 22 South Molton Street, London W1).

7–8 July, **Symposium on Nutritional Aspects of Food and Agricultural Policies in the UK**, Reading (M. R. Turner, Hon. Programmes Secretary, Department of Physiology and Biochemistry, The University, Southampton).

11–14 July, **People and Computers, Computers and People**, Colchester (M. Brady, Department of Computer Science, University of Essex, Wivenhoe Park, Colchester, Essex).

11–15 July, **Conference on Comprehensive Cancer Control**, Copenhagen (WHO, Regional Office for Europe, 8 Scherfigsvej, Copenhagen, Denmark).

11–15 July, **ECM and ECCM for Digital Communications**, Washington, DC (Continuing Engineering Education, The George Washington University, Washington, DC 20052).

11–15 July, **2nd International Conference on The Organometallic and Coordination of Chemistry of Germanium, Tin and Lead**, Nottingham (J. F. Gibson, The Chemical Society, Burlington House, London W1).

12 July, **Symposium on X-ray Diffraction and Spectrometry**, London (The Meeting's Officer, The Institute of Physics, 47 Belgrave Square, London SW1).

12–14 July, **Recognition Marking of Animals in Research**, London (Marking Conference secretary, RSPCA Headquarters, Causeway, Horsham, Sussex).

14–15 July, **Processing, Structure, Properties and Performance of Polymers**, Nottingham (The Meeting's Officer, The Institute of Physics, 47 Belgrave Square, London SW1).

17–22 July, **3rd International Conference on Cyclic Nucleotides**, New Orleans, Louisiana (W. J. George, Tulane University School of Medicine, New Orleans, Louisiana 70112).

18–22 July, **Symposium on Limitations and Potentials of Biological N₂ Fixation in the Tropics**, Brazilia (J. Dobereiner, EMBRAPA, Km 47, Seropedica, 23460, Rio de Janeiro, Brazil).

21–22 July, **Symposium on Plants and the Atmosphere**, Lancaster (Royal Meteorological Society, James Glaisher House, Grenville Place, Bracknell, Berkshire).

23–26 July, **7th International Congress-Conjugation Reactions in Drug Bio-transformation**, Turku, Finland (A. Aitio, Department of Physiology, Kiinamyllynkatu 10, 20520 Turku 52, Finland).

28 July–26 August, **5th International Course on Techniques in Molecular Biology**, Santiago, Chile (J. A. Allende, Faculty of Medicine, University of Chile, Casilla 6671, Santiago 7, Chile).

2–5 August, **Working Group on Evaluation of School Health Programmes**, Bucharest (WHO, Regional Office for Europe, 8 Scherfigsvej, DK-2100 Copenhagen, Denmark).

5–8 September, **2nd International Symposium on Acetylenes, Allenes and Cumulenes**, Nottingham (J. F. Gibson, The Chemical Society, Burlington House, London W1, UK).

5–9 September, **Course in Fluorescent Microscopy**, Oxford (Royal Microscopical Society, 37/38, St Clements, Oxford, UK).

6–8 September, **International Research Symposium on New Processes of Waste Water Treatment and Recovery**, London, UK (The Conference Secretary, Society of Chemical Industry, 14 Belgrave Square, London SW1, UK).

6–10 September, **27th Session of the Regional Committee for Europe**, Munich (WHO, Regional Office for Europe, 8 Scherfigsvej, DK-2100 Copenhagen, Denmark).

13–16 September, **Symposium on Unconventional Photographic Systems**, Oxford (Royal Photographic Society of Great Britain, 14 South Audley Street, London W1, UK).

13–16 September, **4th International Symposium on Nuclear Quadrupole Resonance Spectroscopy**, Osaka, Japan (4th International NQR Symposium, Faculty of Science, Osaka University, Toyonaka 560, Japan).

14–16 September, **3rd National Quantum Electronics Conference**, Southampton (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

16 September, **3rd North West Regional Symposium of the Chemical Society**, Salford (H. Suschitzky, Department of Chemistry and Applied Chemistry, University of Salford, Salford, Lancashire, UK).

18–23 September, **2nd X-ray Powder Diffraction Conference**, Exeter (The Conference Secretary, Phillips Analytical Department, Pye Unicam Ltd, York Street, Cambridge, UK).

19–22 September, **International Conference to Mark the 50th Anniversary of the Discovery of Electron Diffraction**, London, UK (Institute of Physics, 47 Belgrave Square, London SW1, UK).

19–23 September, **2nd International Symposium on the Biological Oxidation of Nitrogen in Organic Molecules**, London, UK (J. W. Garrod, Chelsea College Annexe, 271 King Street, London W6, UK).

19–24 September, **4th Biennial Congress of the International Society for the Study of Behavioural Development**, Pavia, Italy (The Institute of Psychology of the Faculty of Letters and Philosophy of the University, Corso Strada Nuova, 65 27100—Pavia, Italy).

19–24 September, **10th World Energy Conference**, Istanbul (Secretary General of the World Energy Conference, 5 Bury Street, St James's, London SW1, UK).

19–24 September, **8th International Symposium on Earth Tides**, Bonn, FRG (Werke-und Verkehrsamt der Stadt Bonn, Postfach 864, D-5300 Bonn-Bad Godesburg, FRG).

20–21 September, **Population Control by Social Behaviour**, London, UK (Institute of Biology, 41 Queen's Gate, London SW7, UK).

20–22 September, **1977 Autumn Meeting of the Chemical Society**, Aberdeen (The Chemical Society, Burlington House, London W1, UK).

26–28 September, **On-Line Surveillance and Monitoring of Process Plant**, London, UK (Conference Secretariat, 'On-Line Surveillance and Monitoring

of Process Plant', Society of Chemical Industry, 14 Belgrave Square, London SW1, UK).

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Energy, Mines and Resources Canada. Geological Survey of Canada. Bulletin 265: Notes on the Petrology of Nepheline Gneisses Near Mount Copeland, British Columbia. By K. L. Currie. Pp.28. \$4.80. Paper 75-12: The Hay River Formation and Its Relationship to Adjacent Formations, Slave River Map-Area, N.W.T. By G. K. Williams. Pp.17. Paper 75-27: Geology and Engineering Characteristics of Surficial Deposits, Montreal Island and Vicinity, Quebec. By V. K. Prest and J. Hode-Keyser. Pp.27. \$4.80. Paper 76-23: Terrain Characterization and Evaluation—an Example from Eastern Melville Island. By D. M. Barnett, S. A. Edlund and L. A. Dredge. Pp.18 \$3.60. Paper 76-25: VLF Mapping of Geological Structure. By W. M. Telford, W. F. King and A. Becker. Pp.13. \$3. (Ottawa: Geological Survey of Canada, 1976 and 1977.) [124]

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Report of the Council for Tobacco Research—USA, Inc., for 1976. Pp.86. (New York: The Council for Tobacco Research—USA, Inc., 110 East 59th Street, 1977.) [274]

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nature

16 June 1977

Walking on two legs

THE regular two-way flow of scientists and technologists that has now been established between the People's Republic of China and the rest of the world has led to some fascinating discoveries about the way in which Chinese research is done and how it is managed. Now the Organisation for Economic Co-operation and Development (OECD) has produced a large-scale document, *Science and Technology in the People's Republic of China*, which attempts to produce an integrated viewpoint on the nature and evolution of China's science and technology system. It should be widely read, particularly by those just about to embark on a visit to China.

OECD's Committee for Scientific and Technological Policy has, of course, been analysing national science policies for many years, generally at the invitation of member countries who have, during the course of the analysis, staged a 'confrontation' with the OECD examiners. In this case, no Chinese were present at the seminar of thirty-odd science-policy experts and sinologists from a dozen countries out of which this report arose. But although it is the work of outside observers and it lacks some detailed information (which probably is all but inaccessible in China itself), the document does most clearly outline the dilemma of organising science and technology in an immense, partly developed nation with major demands both on the urban and rural sector and with a fluctuating but potent ideology in the driving seat.

China seems to live perpetually under the influence of two opposing forces; walking on two legs is how the Chinese describe their attempts to come to terms with these polarities. Elitism and egalitarianism; old and new; 'expertness' and 'redness'; theory and practice; ideology and technology; Mao and non-Mao. And even these poles are very mobile and slippery, as Simon Leys recently demonstrated in the *New York Review of Books* with a mischievous juxtaposition of some of the late Chairman's mutually-contradictory thoughts. Perhaps the fundamental question is whether China is governable at all—if not governed firmly, it is in danger of falling into anarchy and fragmentation. If governed firmly, it is in danger of being strangled by the very

growth of bureaucracy this tends to bring with it', was the way one participant in the seminar put it.

Small wonder then that China's recent scientific and technological past should be marked by grotesque ups and downs. During the Great Leap Forward and the two preceding years (1956–60), university enrolments more than doubled, and the science budget more than quadrupled. But the country wasn't yet equipped to use its graduates and in the next few years enrolments declined by about a third as 'realism' took over and smaller growth was accepted. But then came the next violent change, the Cultural Revolution of 1966. This time universities were all but annihilated for a period of a couple of years as the higher educational system came under attack on all fronts, including its alleged discrimination against workers and peasants. In the recovery period of the past few years students now go through at least two years of manual labour in rural areas before proceeding to higher education.

Science itself was relatively well protected at the beginning of the Cultural Revolution, by the '16-point Directive', which stated 'we must take particular care of scientists . . . who have made great contributions. Efforts should be made to transform slowly their world outlook and their style of work'. This protection did not last, as the pressures for egalitarianism and Chairman Mao's philosophy bit more deeply ('Intellectuals specialising in engineering are better, because they are in touch with reality. Pure scientists are worse, but are still better than those who specialise in the arts. Students of history, philosophy and economics have no concern with studying reality—they are the most ignorant').

The results of these upheavals are now widely known to a growing band of people. And amongst these there has been strong criticism of reduced academic standards, together with muted approval for the science-for-the-people approach. But in the past few years the tide seems again to have been moving slowly the other way, and probably with Mao's death (which came after this seminar had occurred) will move a little quicker. China may start again to walk even more evenly on two legs; this report will provide an excellent guide for the onlookers. □

Persepolis and Miami Beach

Alvin M. Weinberg, of the Institute for Energy Analysis at Oak Ridge, Tennessee, comments on the evils of energy from fossil fuels and nuclear fission.

MIAMI Beach and ancient Persepolis must have as little in common as any two places in the whole world. Yet in the past three months each was the site of a scientific meeting that in an unsuspected way impinged on each other and possibly on our whole future. At Miami Beach 80 climatologists, ecologists, oceanographers, energy soothsayers, and scientific administrators met to discuss carbon dioxide in the atmosphere. At Persepolis, 500 nuclear people met to discuss transfer of nuclear technology. I attended both meetings.

Miami Beach marked the first time that most of the United States workers on the CO₂ problem, plus a sprinkling of European experts, gathered to decide what might be done about the great uncontrolled experiment—the effect of the accumulation of CO₂ in the atmosphere on climate. The atmosphere contains about 700×10^9 tons of carbon as CO₂; its present concentration is 330 parts per million (ppm). The concentration has been increasing at about 1 ppm per year during the 20 years that Professor Keeling of the Scripps Institute of Oceanography has been monitoring it. This increase corresponds to about one-half the total CO₂ thrown into the atmosphere by the burning of fossil fuels.

How much of the increase in CO₂ really comes from burning fossil fuel, how much from clearing of the world's forests? The oceanographers were adamant: there was no room in the oceans to accommodate a contribution from forest clearing that was a significant fraction of the contribution from fossil fuel. The ecologists were less adamant: from what we know about forest cutting, we cannot say whether the biosphere is a net source or a net sink of CO₂. More experiments and observations are needed.

If one assumes the CO₂ increase is caused by the burning of fossil fuel, what is the likely ultimate level of atmospheric CO₂? Since removal of CO₂ to the deep ocean requires 500–1,000 years, the concentration of CO₂ would double in about 300 years, even at the present rate of burning fossil fuel. But if the world's use of fossil energy increases—say sixfold by 2050—then the CO₂ concentration might double in 75 years.

The climatologists, largely basing their conclusions on the Global Circulation Model of Manabe and Wetherald, came close to predicting that a doubling of CO₂ concentration would cause an unprecedented warming of the climate: about 2 °C average, 8–10 °C at the poles. Moreover, the usually invoked stabilisers, especially clouds, did not seem to change these estimates significantly.

What, if anything, can one do about CO₂? Schemes ranging from dumping the CO₂ directly into the deep ocean (a suggestion of C. Marchetti) to planting a trillion trees (F. Dyson) were described. But mostly there was an air of helplessness. If CO₂ is as big a problem as many now suspect, we simply may have to limit our use of coal and other fossil fuels or live with whatever consequences may arise. Prudence dictates that in any case we keep open the world's energy options—solar, fission, fusion, conservation.

Which brings me to Persepolis. The most important paper discussed there was not even on the agenda: it was President Carter's nuclear policy, which was announced on the eve of Persepolis, and which completely dominated the proceedings. How could it be otherwise? Persepolis aimed at facilitating transfer of nuclear technology; President Carter's policy, though domestic, was largely aimed at minimising proliferation and therefore at limiting the

transfer of certain nuclear technologies. No wonder most of Persepolis reacted as though mortally wounded.

Did Persepolis react too strongly? I think it is too early to say. After all, President Carter's proscription of plutonium recycling in light water reactors (LWRs) is simply a continuation of President Ford's policy. It goes beyond the Ford policy in deferring the Liquid Metal Fast Breeder Reactor (LMFBR). Now plutonium recycling in LWRs is only marginally economic in the US; and although it is unclear how the nuclear enterprise will permanently dispose of its wastes unless some reprocessing is allowed, I don't think it was the continued US proscription of plutonium recycling in LWRs that mainly bothered Persepolis.

Nor did President Carter's call for investigation of breeders other than the LMFBR bother me. I have long believed that the entire world locked itself into the LMFBR before it really investigated the alternatives. The real question to my mind is whether the President's policy represents a firm commitment to develop *some* breeder or was only the first step in soft-peddalling all breeder development.

The basic concern at Persepolis was that the new policy may betray an underlying inclination to have done with nuclear energy: let LWRs run their course, and because of the danger of proliferation, do nothing serious about the breeder. And if the breeder is not developed, nuclear energy will wither as our low-cost uranium is used up.

But what then of the necessity of keeping options open, a necessity made urgent at Miami Beach? At Miami Beach the alarm-sayers sense the end of fossil fuels because of CO₂. At Persepolis the alarm-sayers sense the end of nuclear energy, crucified on a cross called proliferation.

I would like to believe that both alarm-sayers are overreacting: that President Carter's policy will really lead to the timely development of several breeders, and that CO₂ somehow isn't the Sword of Damocles pictured at Miami Beach. But these are hopes, not necessary realities.

To weigh proliferation against climatic change is surely beyond human wisdom. Yet there is an asymmetry in these catastrophes, assuming they really exist, that suggests a proper course. The CO₂ catastrophe, if it indeed exists, will occur even if the fossil fuel system works *properly*: it can hardly be avoided short of drastically reducing our use of fossil fuels. Proliferation, by contrast, could be, though not necessarily, a consequence of an *improperly* operating breeder system, that is, one in which technical and institutional barriers to proliferation have failed.

Proliferation stemming from breeders can in principle be forestalled by proper design, both technical and institutional; a CO₂ catastrophe (assuming it exists) hardly can be forestalled except by changing to a non-fossil energy system. It therefore seems clear to me that we turn away from serious development of breeders at great peril. I hope my concerns are without merit, and that the President's policy will lead to greater, not lesser, emphasis on the practical achievement of proliferation-resistant breeder systems. □



Plant a tree and save the world?

An aid to development?

How can science be made to help poor countries?

Alastair Hay looks at part of the UK and Commonwealth effort

TWO international conferences in the past month have tried to resolve the problems involved in dividing the spoils of the world between rich and poor. The first meeting, the Conference on International Economic Cooperation or 'north-south' dialogue in Paris, failed to live up to expectations. The second, the Commonwealth Heads of Government meeting in London last week, had less expected of it. Both have hitherto recognised one way or another a role for science and technology in improving the relationship between rich and poor countries. The difference is that the Commonwealth possesses the means to do something about it.

The final communique from the Paris meeting indicated that the ultimate goal sought by poor countries of a new international economic order was a long way off. No figure was put on a common fund to help stabilise raw material prices, a \$1,000-million aid package fell far below Third World needs, and a general moratorium on Third World debt repayments was refused; on energy the West's goal of discussions between oil producers and consumers was not achieved. But with the countries still talking to each other it was not an unmitigated failure.

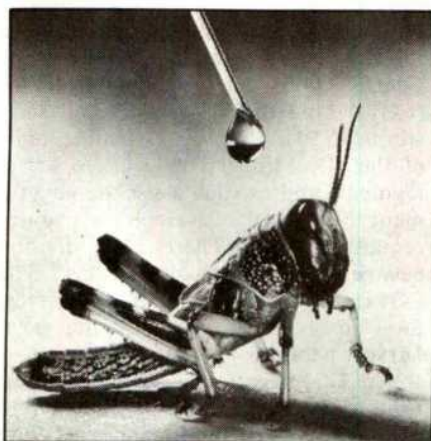
Some further progress came from the Commonwealth meeting. And in line with the view that the developed countries will need to provide more aid, the UK Ministry of Overseas Development (ODM) announced a higher contribution to the Commonwealth Fund for Technical Cooperation (CFTC). Through this multilateral development aid fund financed by all Commonwealth governments, the Commonwealth Secretariat supports activities undertaken by such organisations as the Commonwealth Science Council and provides technical assistance in scientific fields to Commonwealth governments. The Commonwealth Science Council (CSC) is the main agency for promoting scientific collaboration between Commonwealth countries, and the increase will have pleased the Commonwealth Secretary General, Shridath Ramphal. In a recent symposium in Brussels on science strategy for Europe he voiced the opinion that "structural change in the world's economic system is the pre-condition of real development". To achieve the new international economic order which the poor countries were seeking, he said, "an important, indeed an essential and integral element,

would be a new, enlightened and dynamic role for science and technology".

The Commonwealth's role in this sphere is not well known, not least because of its minuscule size. The CSC's aim is to increase Commonwealth countries' "national capability to use science and technology for economic and social development", an onerous task for an organisation with a 1976 budget figure of only £100,000 provided by its 26 member countries and £20,000 for project funding by the CFTC. The CSC had its origins in the informal scientific collaboration between Australia, Canada and Britain prior to and during the Second World War. A conference convened by the Royal Society in 1946 placed this collaboration on a more formal basis, and the Council expanded thereafter to incorporate other Commonwealth countries and had several name changes before finally adopting its current title in 1975. By 1972 with the formation of national science research councils in many developing countries most governments were able to define their development problems more specifically, and this helped the CSC to communicate more effectively with them and especially with the research councils.

The Commonwealth Geological Liaison Office, which publishes reports and a news letter containing information on minerals and geology of interest to Commonwealth countries, and the Permanent Commonwealth Collection of Microorganisms, which promotes the expansion of culture collections and their wider use within the Commonwealth, both function under the aegis of the CSC. Further Commonwealth science collaboration is promoted by the Commonwealth Agricultural Bureau and Institutes which disseminate research information in specialised fields of agricultural science. Two more technologically sophisticated science agencies of the Commonwealth are the Commonwealth Advisory Aeronautical Research Council and the Commonwealth Consultative Space Research Committee. And the Commonwealth Foundation exists to provide grants for specialist conferences, study and research visits, bursaries and lectures.

Like all organisations, the CSC has had its difficulties. A Working Group report in 1974 expressed the view that CSC's work should be expanded but with more coherence than was evident



Testing pesticides at the COPR

in the past; in this way it might get across to more scientists in the Commonwealth. Following this gentle upbraiding the CSC streamlined and concentrated its operations. Those of prime concern include standards for engineering and industrial products; industrial research and training; scientific communication and management; mineral exploitation; the technological infrastructure of rural communities; non-conventional energy resources; and preservation and storage of food.

It is a list to daunt many development scientists. The CSC's Executive Secretary since 1972, Gwyn Thomas, who combines his role with that of Scientific Adviser to the Commonwealth Secretary General, agrees that the CSC has a demanding brief, but stresses that its purpose is rather to act as a catalyst than to run specific development projects. The CSC is working towards coverage by organising regional conferences on one or other of these topics, with several interested countries participating. These meetings are held to define common problems and to assist with the formulation of a joint R&D strategy to solve them. It is also encouraging greater sharing between countries of facilities for industrial training. Member countries train technologists in the use of quality standards and there is a move to promote regional harmonisation of standards. As the CSC sees it, quality standards are essential if exports from developing countries are to be sold in highly competitive Western markets.

Crucial concern

The subject of food preservation and storage remains one of crucial concern to the CSC. In view of the Council's limited resources in this field, it is again restricted to exhortation and organisation of meetings. But two UK organisations echo CSC interest in this field and are able to participate actively in development programmes; as a result they provide much relevant

scientific data for food preservation in the developing countries. They are the Centre for Overseas Pest Research (COPR) and the Tropical Products Institute (TPI). Both are scientific units of the UK Ministry of Overseas Development, and as such assist the government of any developing country requesting help. There is a division between their respective roles. The COPR is concerned with pre-harvest food losses, the TPI with the post-harvest period.

The COPR is the newer of these two units. Formed in 1971 by the merger of four ministry units concerned with locust, tropical pesticide and termite research, it started life with its component units having over 60 years' experience in overseas pest control. With this background and the resources available to it—the unit's R&D budget for 1976 was £816,000—the Deputy Director, Tecwyn Jones, found it logical for COPR to "concentrate on pre-harvest loss control and public health fields involving insect vectors".

Jones's experience of pest and insect vector control in the tropics where harvests and health often cannot be guaranteed without pesticide use makes him conscious of the continuing need for chemical control. The objective is to guarantee farmers' crops in the developing world and to protect the health of the workers in the field. However, the COPR is fully conscious of the dangers and shortcomings of persistent insecticides and recognises the urgent need to search for alternative ways of control and use available insecticides more prudently. To this end it is promoting the use of ultra-low volume spraying of pesticides in the tropics. With this technology, sprays effective for the control of tsetse fly can be reduced from 1,000 grams of pesticide per hectare to as low as 6 grams per hectare. The COPR is presently investigating the use of synthetic pyrethroids as alternatives to such chlorinated hydrocarbons as endosulphan, DDT and dieldrin.

Control of insect pests in the future seems in the COPR's view to lie in the use of a combination of insect pheromones—to disrupt mating and reproduction habits—and viruses which are specific to certain pests. Natural parasites and predators may also have a role to play. A COPR programme to test pheromone/virus control has begun and field trials are to be conducted next year in the Mediterranean area, and in East and Central Africa.

On an international scale the COPR collaborates with UN agencies like FAO and WHO. It acts as a reference centre for FAO on locust migratory patterns, and research in the unit on plant resistance to specific insect pests could provide genetic information of

relevance to plant breeders. The unit serves as an international reference centre for WHO for the evaluation of new insecticides for disease vectors such as malaria mosquitoes and tsetse flies, and has an intensive programme to test new molluscicides on the bilharzia parasite.

Broader role

The larger and older of the ODM units is the TPI. With a history going back to 1894 it has amassed considerable expertise in the field of post-harvest processing, preservation, storage and marketing of tropical products. For most of its existence the institute ministered specifically to the needs of the Commonwealth. However, with its incorporation into ODM in 1965, it was able to participate in work directed to the developing world as a whole.

Malcolm Thain is a deputy director of the TPI and is involved in directing the institute's R&D work, which in 1976 had a budget of £1,058,000. He says that the 1975 ODM White Paper 'More help for the poorest' confirmed the TPI in its basic work and that it is generally acknowledged that for Third World crops there is insufficient information on where the losses actually occur in the chain from harvesting to the consumer.

Some of the TPI's research programme is therefore directed towards identifying the weak points in this chain. Inevitably, unfortunate side effects of the green revolution have been identified: varieties of maize especially bred for yield often prove to have poor storage qualities, for example. This has led many Third World farmers to revert to their own lower yielding but better storing varieties. The TPI is thus directing some of their efforts towards the problem of storage of high yield crops, genetic breeding for 'storage properties' as well as yield, and low cost technology for assisting farmers to both dry and store crops. For instance, cassava has a storage life of a few days before

decay sets in. TPI scientists have found that a simple earth clamp, similar to that used for storing potatoes, will preserve the crop for up to 3 months.

Other TPI research efforts are concerned with insect pests, again using pheromones as well as fumigation methods, and quality control trials on those foodstuffs of developing countries which have an export potential. One TPI department of increasing relevance to developing country needs is the Industrial Development Department (IDD) near Culham, responsible for most of the ODM research effort on appropriate technology.

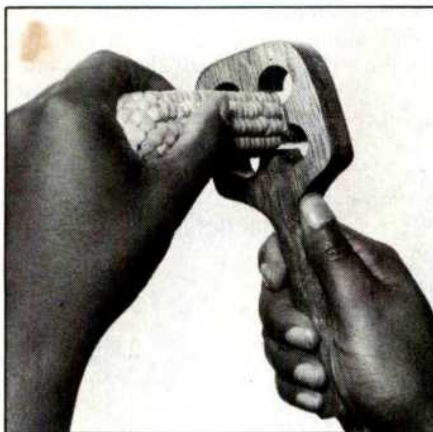
David Adair, the head of IDD, is one of those enthusiasts the field of appropriate technology seems to generate. He laments the fact that there is still insufficient grasp of the essential elegance and simplicity of some of the technologies the TPI is working on. Often because they lack the complicated gadgetry of much of today's sophisticated equipment, the items tend to be regarded with a certain air of condescension. However, Adair is not too concerned. Increasing orders for IDD's products is sufficient confirmation of the need for this technology in the developing world. A few of the unit's developments include a still for bay oil extraction which will run on leaves as fuel, a cashew nut processing plant, sunflower seed, cotton seed and groundnut decorticators, simple milling devices, and maize and coconut shredders. IDD places great emphasis on fuel conservation and devotes much attention to alternative sources of fuel like coconut charcoal and to non-conventional building materials.

Demand problem

Adair's problem—he receives more requests for assistance than he can hope to provide—is common to the TPI as a whole as well as to COPR and CSC. All three concerns publish extensive information about their activities and rely to a considerable extent on publicity to get their message across. But the resources required to meet the needs of the developing world are far greater than those possessed by these three organisations, or indeed by the international organisations or charities.

Development programmes in Third World countries embrace not only science and appropriate technology but sociology, ethology, medicine and economics as well. Integration of these disciplines into aid programmes is now an accepted practice, but personnel and resources are still too limited.

The solution is succinctly put by the Commonwealth Secretary General: "It is not lack of knowledge about what needs to be done, but the will and commitment to do it". □



Corn off the cob

ISRAEL

Exploiting an advantage

Nechemia Meyers learns of the likely prospects for science and technology under Israel's new government

"WE in the Likud realise that resource-poor Israel has a comparative economic advantage in only one sphere—science and technology—and so we will do everything we can to ensure that this advantage is exploited to the fullest possible extent". So says Professor Moshe Arens, the unofficial science spokesman for Israel's new Likud Government.

The Likud's concern with science and technology may stem in part from the fact that it has four men with a technological background in its 45-member Knesset delegation. Apart from Arens they are Professor Joseph Rom of the Haifa Technion's Department of Aeronautical Engineering, Mr Gustav Badian, Secretary-General of the Engineers' Union, and Mr Yitzhak Moday, who has a degree in chemical engineering but specialises in economic subjects.

The Likud does not favour the establishment of a separate Ministry of Science and Technology, however. This, it believes, would be counter-productive. "Instead of pigeonholing these subjects", declares Arens, "we must ensure that every single ministry

is concerned with science and technology, and particularly with exploiting Israel's own technological potential."

In the defence sphere, for example, Arens readily admits that in present circumstances Israel would still be dependent on certain American components, but argues that if there should be an American embargo on the supply of these components, they could be purchased elsewhere or produced at home. He points with pride to the fact that Israel's highly effective missiles, which he helped to develop, have brought in tens of millions of export dollars, and he is certain that many more high-technology civilian products could do the same.

"We are already selling a whole range of sophisticated items ranging from pharmaceuticals and laboratory chemicals to computerised whole body scanners for hospitals and computerised design systems for textiles", he says. "But much remains to be done. Take solar energy, a field in which we have pioneered. Even now Israel uses more solar energy per capita than any other country in the world, and she also sells some solar energy installations overseas. However, if our scientists are given proper encouragement, I'm convinced that they will be able to develop more efficient methods of exploiting solar energy".

According to press reports, Arens

may be put in charge of a ministry or an authority dealing with energy, water, building and other elements of what he calls the country's physical infrastructure. While refusing to comment on these reports, he does stress the Likud's view that the development of Israel's physical infrastructure must be more carefully planned, and its component parts utilised in a more rational manner.

Arens promises that Israel's R&D facilities, whether in agriculture, industry or some other sphere, will get all the support they require both from the Likud government and from private investors, whom the Likud believes it can encourage by creating a better climate for investment. However, the universities, which are almost entirely dependent on public funds, may be in for a tough time—after already suffering a drop of 30% in real income since 1973.

While Arens declines to estimate how much support the universities can expect, he does note that the Likud will have to slash the government budget if it is to reduce inflation (now running at over 40% a year). This means that at least for the next few years "short term targets will have to be given priority over longer-term targets". Being a faculty member himself, Arens doesn't lack sympathy for Israel's institutions of higher learning. But his presentation of the Likud's priorities gives them clear cause for concern. □

BRITAIN

Publish and be dammed

In the continuing debate over Britain's energy policy there is still no shortage of information being published. Chris Sherwell reports

WHILE final preparations were being made for the opening of the Windscale inquiry this week, one of the chief opponents of the controversial project was with impeccable timing publishing a new book seriously questioning Britain's commitment to an electro-nuclear future. It was not the only publication on the energy front, however. The UK Department of Energy (DEN) was busily announcing another set of findings regarding its strategy for developing alternative energy sources, this time for tidal power and particularly the Severn barrage. Four reports were published on this matter alone. The DEN also released a review, prepared at the end of last year, detailing the country's energy policy options.

The inquiry into British Nuclear Fuel Ltd's (BNFL) application to develop land at Windscale for an oxide fuel processing plant was due to open on Tuesday morning in nearby Whitehaven, Cumbria, before Mr Justice Parker, the inspector, and two assessors, Sir Edward Pochin and Professor Sir Frederick Warner. BNFL submitted an outline planning application to the local authorities in February. In accordance with his announcement last December, Mr Peter Shore, Secretary of State for the Environment, ordered the public inquiry to allow examination of health, safety, environmental and planning aspects.

The inquiry, which is expected to last some three months, is widely regarded as a critical test for the future of nuclear power in Britain. On it also hangs the fate of deals worth hundreds if not thousands of millions of pounds for reprocessing other countries' ir-

radiated oxide fuel. BNFL's statement of case, circulated in May, emphasises the conservation and waste control benefits of reprocessing and the economic advantages of taking on overseas business. Opponents, who include Friends of the Earth, the Conservation Society and others, have retained expensive legal counsel and sought to raise funds publicly.

The inquiry was expected to begin with brief opening statements on behalf of BNFL and the various opponents before proceeding later in the week to BNFL's detailed presentation. The basis for the sort of arguments the corporation will then face is contained in *The Fissile Society* by the FOE's energy specialist, Walt Patterson, and published last week*. With the inquiry almost certain to spill beyond the immediate issue of the reprocessing plant itself, the book is a useful articulate distillation of the case against a nuclear future that neatly ties together a historical perspective and the prevailing sense of urgency.

No such urgency is apparent in

connection with the long-standing and increasingly widely supported idea of a barrage across the Severn Estuary in the west country that would harness the energy from the tides. At a press conference to announce publication of results of three special studies relating to the barrage† and another more general report from the DEN§, Dr Walter Marshall, the department's chief scientist, could offer no date for a decision from the DEN's Advisory Council on Research and Development (ACORD) on the next step, a large-scale £1–2-million feasibility study.

This will not have encouraged people like Patterson, who argued strongly last week for a redistribution of Britain's energy R&D effort, and Arthur Palmer, the chairman of the House of Commons Select Committee on Science and Technology, from which a report on the barrage is due out in the coming months along with a separate report on other alternative energy sources. According to a spokesman for NEDECO, the Dutch engineering group which conducted one of the studies, the earliest that work could begin with a decision now is 1982, to allow for four years of preliminary study. Sixteen years later the project could be complete, at a cost variously estimated (depending on whether it is a 'single basin' or 'double basin' scheme) between £2,400 and £3,100 million at today's prices—excluding the turbines.

ACORD is now considering all the reports, which come 12 days after a one-day symposium on the subject in London. None of them examines the environmental effects of a barrage; that, says Marshall, would have proved both difficult and expensive. They were designed to consider, first, the influence of various barrage schemes on tidal levels and currents, and secondly, the technical feasibility of a barrage. The Hydraulics Research Station (HRS) study speaks cautiously of an increase in spring tidal ranges seaward of the barrages of about 1.4 m, but NEDECO speaks of a decrease of similar order. General changes in currents, however, would be small, but navigation, flooding and drainage would all be affected. On the positive side the Institute of Geological Sciences report finds the seabed at the barrage area uncovered by drift and with bedrock exposed. NEDECO's finding is the most positive: the complex project is technically feasible. The problems, therefore, are now economic and political. □

**The Fissile Society*, Walter C. Patterson, Pp. 117 (Earth Resources Research Ltd., London, 1977), £1.50.

†*Severn barrage study*, HRS, £2; *Severn tidal barrage scheme: pre-feasibility study of the closure of the estuary*, NEDECO, £4; *Geology of the Severn barrage area*, IGS, £1. (Obtainable from DEN, Millbank, London).

§*Tidal power barrages in the Severn Estuary*, Energy Paper No 23 (HMSO, £1.50). Also released, *Energy Policy Review*, Energy Paper No 22 (HMSO, £1.75).

SWEDEN

A developing nuclear policy?

Wendy Barnaby reports from Stockholm on recent developments concerning Sweden's nuclear programme

THE Swedish government is to give credit guarantees for the continued building of the Forsmark 3 nuclear reactor but not for Oskarshamn 3. In a classic case of a 'no-decision decision'—now the norm for the government's handling of the nuclear energy issue—the energy minister, Olof Johansson, said recently that about \$30 million would be guaranteed for Forsmark 3 while the reactor's owners agreed on the pace at which building should be continued. In a couple of months, when the negotiations were expected to be finished, the government would reconsider the question of more guarantees. He himself would not comment on the reactor's development, saying that he did not want to pre-empt the owners' negotiations.

The government gave no reason why guarantees to Oskarshamn had been refused, but a spokesman for the company said that there were two probable reasons. One is that, whereas the Forsmark reactors are 75% state-owned, Oskarshamn is a private company. In order to continue building their reactor without state financial guarantees, the management of Oskarshamn will probably have to approach ASEA-Atom—which is 50% state-owned—for help. The state could therefore gain some indirect control over the project.

The other reason is that the State Power Board, the largest owner of Forsmark's reactors, employs 2,000 workers who have been building Forsmark 2 and who would be unemployed if they could not now build Forsmark 3. The Oskarshamn practice, on the other hand, is to contract out its work. The government could therefore have been hoping to avoid visible unemployment.

Unemployment is very much on the minds of the industrial workers dependent on the reactors for their jobs. Six trade unions have sent a joint letter to the energy minister demanding an immediate, clear decision on the short and long term prospects for the construction of reactors. It is highly unlikely that they will get it. All they can hope for is clearer guidance in autumn 1978, when the government plans to present proposals for a new energy policy up to 1990.

The management of Oskarshamn is planning to go ahead with the construction of the third reactor, even

though it will be delayed now by up to two years. They reason that, if they can arrange their own finances, there is no reason why they should not in future be treated differently from Forsmark 3. The owners of each reactor will, under the law stating the conditions on which new reactors may be built, have to present the government with concrete proposals for the 'completely safe' storage of unprocessed fuel or of highly radio-active waste if the spent fuel is to be reprocessed; and reprocessing agreements will also have to be presented.

Although the Centre Party was elected to the government largely on its promises that it would stop nuclear power in Sweden, it has so far shown no particular hurry to do so. And if it is prepared to entertain the possibility of contributing to Forsmark 3, the Oskarshamn management evidently doubts that it will put in jeopardy the \$330 million-worth of orders placed last year with different sections of local industry for the components of Oskarshamn 3.

There is no more certainty about the reprocessing and storage of spent nuclear fuel than there is about the building of the reactors themselves. If the Ringhals 3 reactor produces any spent fuel before the end of 1979, it will be sent to La Hague in France for reprocessing. Otherwise, according to the technical director of the State Power Board, it will have to be stored without reprocessing. This solution may also have to be adopted for the Forsmark 1 reactor, which so far has no reprocessing agreement for the spent fuel it should begin to produce next year. The State Power Board envisages waste being stored in a central depot until 1995, by which time it evidently hopes that other solutions will have become available.

A second round of test drilling, designed to examine the quality of rock near Forsmark, is now being started by the Swedish Geological Survey on behalf of the companies building reactors. Preliminary results from the first tests, near Oskarshamn, were in general good, showing hard rock with low permeability—the sort of rock that would be suitable for storing nuclear waste. According to a spokesman for the nuclear companies, storage of processed, vitrified waste and the direct deposition of encapsulated fuel elements will be considered. The main geological reports are expected to be ready by 1 October this year, but supplementary reports will be issued during 1978. □

IN BRIEF

Orlov appeal

Almost 600 French physicists have signed a declaration in defence of Professor Yurii Orlov, founder of the unofficial Moscow 'Helsinki monitoring group'. This was announced this week at the close of the 'Orlov hearing', a mock trial staged at the Institute of Physics in London on the eve of the Belgrade review of the implementation of the Helsinki agreements.

Orlov was arrested on 10 February and is being held incommunicado in the Lefortovo prison in Moscow. He has not yet been formally charged, but it seems likely that when a charge is made it will be concerned with the activities of the monitoring group under the articles pertaining to anti-Soviet propaganda.

The case for the defence, presented in London by John Macdonald QC, analysed the 19 major documents on Soviet violations of the Helsinki agreements, maintaining that the content of the documents was true, and believed

by the members of the group to be true. "Witnesses for the defence" included the mathematician Leonid Plyushch, Vladimir Bukovskii, and Marina Voikhanskaya, a psychiatrist who refused to cooperate with the Soviet misuse of psychiatry for political ends.

Employment prospects

The total number of scientists and engineers employed by private industry in the United States dropped by 5% in the first half of the 1970s, according to a study released last week by the National Science Foundation. The first major survey of scientific employment since 1970, the study also indicated that there has been a major shift in employment patterns, with manufacturing industry reporting a 13% drop and non-manufacturing industries indicating an 11% increase in the number of scientists and engineers they employed between 1970 and 1975. The figures refer to all scientists and engineers working in private industry.

Those directly employed in research and development departments declined particularly sharply, from 372,000 in 1970, to 329,000 in 1975—a 12% drop. Though the survey covered a period of economic recession, the decline in the number of scientists and engineers employed by private industry was unexpectedly sharp. Recent indicators suggest, however, that the scientific job market may be picking up a little as the economy has improved.

Cost-of-living for NSF

Unmoved by protestations that academic science in the United States has been suffering from financial undernourishment, the House Appropriations Committee recently recommended that the National Science Foundation (NSF) should get no more than a cost-of-living increase for its support of basic research next year. The committee, which is the most powerful of the Congressional panels which handle NSF's budget, recommended that

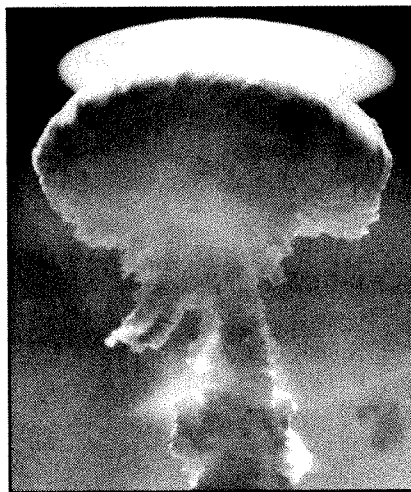
RECENT reports that the United States is planning to build a radically new type of nuclear weapon, designed to kill people by releasing vast quantities of lethal neutrons while causing relatively little blast damage to buildings, may have been a bit misleading. Though details of the proposed weapons are shrouded in secrecy, a number of independent experts have told *Nature* that they are unlikely to differ significantly from the small tactical nuclear explosive already deployed in large numbers.

Funds to begin building the device, for use as a warhead on the Lance short range missile, are contained in budget proposals for the Energy Research and Development Administration (ERDA) which are now being considered by Congress. The first official public reference to the weapon came last March when General Alfred Starbird, Assistant ERDA Administrator for National Security, told a Congressional committee that it would be designed to "reduce the blast effect and get the kill radius you want through enhanced radiation".

All nuclear explosions give off energy in two forms: a blast and heat wave which causes physical destruction of buildings and other structures, and withering radiation in the form of gamma rays and fast neutrons (so-called prompt radiation) which can pass relatively easily through the atmosphere and penetrate solid material, including people.

In addition, radioactive debris from the explosive and from material sucked into the blast will later be deposited as potentially lethal fallout. It has long been known that the

Blasted heat



BACKGROUND

relative importance of these effects depends on a number of factors, including the size of the explosion, the design of the explosive, and the altitude at which it is detonated.

In general, the smaller the explosion, the greater is the relative output of prompt radiation. Design is particularly important: according to a book written two years ago by Theodore Taylor, a former weapons

designer, two explosives with the same explosive yield but with different designs could differ by at least a factor of 10 in their output of fast neutrons. In particular, thermonuclear fusion reactions, which take place in hydrogen bombs, are particularly efficient producers of fast neutrons.

Until recently, the chief goal of weapons designers was to produce high-yield explosives which would destroy targets through blast and heat. These are usually fission-fusion-fission devices in which a fission bomb is used to trigger a fusion reaction which in turn sets off a fission reaction in a blanket of uranium surrounding the device. More recently, design has shifted more toward so-called 'mini-nukes', weapons with small explosive yields which could be used with very accurate missiles to knock out small targets. Again, heat and blast would constitute the chief destructive force of the weapon.

The proposed new 'enhanced radiation' weapon is believed to be a modified mini-nuke, possibly a small fusion device, triggered by a low-yield fission explosion, without the surrounding blanket of uranium. The important point to note is that although the weapon is designed to give out relatively large amounts of neutrons, the heat and blast effects would still be substantial—according to some experts they would probably still account for the largest fraction of the weapon's energy output.

NSF should receive \$844 million next year. The administration had requested \$885 million. Virtually all the reduction would be taken from NSF's basic research programmes, leaving only enough for a 6.5% increase, about the same as the rate of inflation. The committee specifically stated in its report that it believes academic science has fared relatively well in the federal budget in recent years, and that a major boost in support is therefore unjustified. The Senate Appropriations Committee, which is often more generous towards NSF, has yet to act.

Catch quotas for whales

Last week, a meeting of the International Whaling Commission's (IWC) Scientific Committee was the focus for the latest exchanges in a long standing argument over how whale catch quotas are determined. A statement by the Committee, that no species of whale

is under threat from extinction, was challenged by Dr John Beddington of York University, one of Britain's delegates to the conference.

Dr Beddington claimed that the basis of the IWC's estimates of catch quotas, the 'maximum sustainable yield', is founded on a false premise. It states that a maximum harvest will be achieved if whale populations are reduced to 60% of their original size. Dr Beddington, however, believes that at this level whale populations will become unstable and may die out.

It is expected that protesters against whaling will be out in force during the Commission's main conference which begins on 20 June.

Tenerife allegation

The attempt to identify the victims of the world's greatest aircraft accident at Tenerife in March has involved "a travesty of justice and a major insult

to the field of forensic sciences in the United States", according to Bill Eckert writing in the latest issue of *Forensic Science* published by Elsevier, Sequoia. He lists "classical mistakes" made by the Spanish authorities in the investigation. The bodies were removed from the wreckage without marking their location, and were embalmed and placed in coffins before identification was complete. They were examined by local public health and forensic authorities who had little knowledge of the procedure; no dentists were available. The bodies were also removed to another country and to a strictly military base using exclusively non-civilian experts. When other than the best available forensic experts are used, Eckert concludes, questions of a medicolegal nature may never be ascertained. This could affect "one of the greatest legal cases in the history of transportation accidents".

It has long been a standard agricultural practice to improve the soil by ploughing crop residues under ground. When I worked on a farm in the 1920s, a popular two-year rotation was to sow oats and sweet clover (*Melilotus alba*), cut the oats to leave a high stubble, let the clover grow to about eight inches the next spring, plough it under and plant maize. The benefits of such procedures were rediscovered by the late Mr Jerome Rodale, a New York City electrical contractor who turned to farming. He coined the names 'organic farming' and 'organic food' in 1942, and popularised them extensively thereafter. He said in 1964 that one MD in California "has cured four cancer cases by putting them on a 100% organic diet". Mr Rodale also used a machine called a 'Theramak' for taking electrical treatments to give himself "more electrical energy".

The definition 'organic' in terms of food production was given by Mr Rodale's son in 1972 at hearings before New York State Attorney General Louis Lefkowitz:

Organically-grown food is food grown without pesticides; grown without artificial fertilisers; grown in soil whose humus content is increased by the additions of organic matter; grown in soil whose mineral content is increased by the applications of natural mineral fertilisers; has not been treated with preservatives, hormones, antibiotics, etc.

The uncleanness of 'antibiotics' to the faithful followers of Rodale seems paradoxical in view of the fact that these organic compounds are produced by moulds. The term 'etc' makes the definition quite elastic. In the hearings, a New York State governmental investigator reported

that a man selling organic fish stated, verbally and on a placard, that it was caught in the pollution-free mineral-rich Atlantic Ocean. One trembles to think of the horrors of

What is organic?



THOMAS H. JUKES

inorganic fish, but, in any event, 'organic' food is a term coined by Rodale and maintained by his successors and heirs. Its popularity reflects the age-old struggle between reductionism and vitalism. Indeed, the legitimate scientific definition of 'organic' as referring to carbon compounds dates back to the concept that they are produced only by living organisms. This idea was annihilated in 1828 by Wöhler, who made urea from ammonia and carbon dioxide and announced he had synthesised urea "without a kidney, a bladder, or a dog". But vitalism lingers on in the

health food shops where fertilised eggs are sold at a 40% premium because of the "spark of life" imparted by the attentions of the rooster, and it is codified in Oregon, where the Department of Agriculture has issued standards for organic foods, containing "not more than 10% of the allowable residues of any pesticide or other synthetic or artificial substance as established by the FDA". Apparently small amounts of pesticides, like a touch of pregnancy, are permissible. Vitalism has now entered the pages of *Nature*, where we read (12 May) that 'Muck is magic'.

A taste panel at the University of Florida rated a large majority of 'regular' foods as superior to 'health' foods on the basis of "colour, flavour, texture, odour, and general appearance". However, 'organic' foods easily win the cash register battle for their sellers; they cost about 82% more than 'regular' foods in a USDA survey in 1972. Consumers can presumably take spiritual comfort in paying more for lower quality without the possibility of detecting an actual difference by analysis. However, a local case of Q fever is attributed to 'natural fertiliser' used in producing 'organic food'.

When my parents named me Thomas they had not heard the expression "I'm from Missouri", but I believe that they knew of the gospel according to St John, 20 v 24-25. In any case, a doubter I remain, despite Kenneth Mellanby's delight in seeing a headline "Ideas of organic farming being taken more seriously," and his evangelical call to nonbelievers in organic food to repent.

news and views

Biological macromolecules: outmoding the rigid view

from Barry Robson

X-RAY diffraction has been of immeasurable value in the analysis of the three-dimensional structure of biological macromolecules. However, the structures determined by X-ray diffraction of protein crystals in particular are inevitably biased towards a rigid picture because the molecules are 'frozen' by the constraint of crystal packing and periodicity. Crystallographers are therefore often accused of having too rigid a view of the protein as it actually exists in solution, although this is rather unfair since much useful information about flexibility in solution has been inferred indirectly from crystallographic studies. A recent study by McCammon, Gelin and Karplus in this issue of *Nature*, page 585, however, represents the most significant advance to date in quantifying the flexibility of a protein, and their technique can presumably be readily extended to other macromolecules.

In order to understand the possible implications of their work for the re-interpretation of the rigid crystallographic view, it is first useful to consider the theoretical work of investigators such as Cooper (*Proc. natn. Acad. Sci. U.S.A.* **73**, 2740; 1976). It is obvious that the structure in the crystal need not, in principle, be the same as the favoured structure in solution, although the evidence suggests that they are and implies the dominance of the intramolecular forces in the crystal. It is less obvious, if one forgets basic statistical reasoning, that the crystal structure need not be the same as the average structure in solution. Therefore Cooper has emphasised that, in the statistical mechanical distribution of protein conformations, the mode is not necessarily equivalent to the mean. In other words, although the most probable state of the molecule may be the compact globule observed by crystallographers, the properties

measured in solution may reflect the large number of possibilities for different, relatively open structures, each of which has a low probability but which collectively add up to a high probability. Therefore, although it is important to determine the degree of dynamic variability in a macromolecule, it is also important to establish whether or not this dynamic variability can draw the average conformation away from the most probable which is one hopes that observed in the crystal.

In order to resolve these uncertainties, McCammon *et al.* have carried out a computer simulation of the molecular dynamics in which the theoretical equations of motion of all the atoms in the molecule are solved simultaneously over a period of time, the history of the behaviour of the atoms being recorded for analysis. The system used was the small protein pancreatic trypsin inhibitor (PTI), the solvent being neglected except for four water molecules which are strongly bound in the interior. The structure obtained by X-ray crystallography was used as a starting point, and the atomic velocities were increased to yield an average kinetic temperature of 295 K. Analysis of the resulting trajectories revealed "a rich variety of motional phenomena that occur on the atomic level at ordinary temperatures". The internal motion of PTI was seen to be fluid-like and governed by collisions between neighbouring non-bonded atoms, rather than by rotational potentials governed by covalently bonded atoms. The average root mean square (r.m.s.) fluctuation of all atoms was 0.9 Å, although larger displacements of 3 Å or more occurred in some regions on the protein surface.

The value of 0.9 Å relates to the deviation from the average structure obtained by molecular dynamics. When the r.m.s. of all the atoms from the X-ray structure was considered, the deviation rose to 1.7 Å. This qualifies the relation between the dynamic

mean conformation and the statistical mechanical mode observed by X-ray crystallography, although if the answer is quantitatively well defined, it is not conceptually so clear cut. The authors state that the time-averaged structure is "near the X-ray structure but not identical with it".

The results are certainly not bad news for crystallographers: although the shift to the calculated time-averaged structure from the crystal structure might be to some extent an artefact of the models used for interatomic forces and of the neglect of solvent, a more correct treatment could undoubtedly bring about only better agreement. On the other hand, those who favour a flexible picture should also be reasonably happy as the molecule had quite clearly been sampling a series of neighbouring conformations whose average was not identical with the X-ray structure.

Not all of the PTI molecule was shown to be equally flexible. The largest fluctuations, apart from those due to the flexibility of side chains at the protein surface, came from the two ends of the backbone and residues 25-28 which loop out of the molecule. The latter showed particularly interesting behaviour, undergoing changes in shape superimposed on an oscillation with a period of 6 ps. On the other hand, regions of the chain which form into such characteristic local arrangements as α helix and β -pleated sheet have significantly smaller fluctuations than average. However, even these features showed oscillatory behaviour, the β -pleated sheet region participating in a low-frequency 'rippling motion'.

Despite the possible inaccuracy of the potential functions used, the neglect of individual hydrogen atoms, and the neglect of the surrounding solvent, these calculations show fairly clearly that PTI is able to wander through a large number of alternative conformations but is prevented by high energy barriers from wandering too far from the observed X-ray structure. This is

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probably fortunate, because the neglect of the solvent is likely to be increasingly dangerous as conformations less and less like the X-ray structure are sampled.

Molecular dynamics and the nucleic acid controversy?

The potential for future application of molecular dynamics and related techniques seems enormous, and is likely to be as important in the field of nucleic acid structure. Of particular interest would be its application to the current heated debate concerning the 'zipper' model for DNA as an alternative to the classical double helix (Rodley *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 2959; 1976). X-ray diffraction of DNA fibres, as opposed to protein crystals, provides more ambiguous information with which the zipper model also seems to be in good agreement. While the double helix has the advantage of being, in some sense, aesthetically more pleasing, particularly because many other biological macromolecules form helical structures, it has to be remembered that nature does not work under an Arts Council grant. Any DNA structure, however bizarre, is likely to coexist in solution alongside the double helix if accurate energy calculations show it to be of comparable free energy, providing molecular dynamic calculations also show that the alternative structure is kinetically accessible. Although the zipper model appears to contain some bad interatomic clashes, there is a very real possibility that these can

be dynamically relaxed. This could be dynamically feasible; to make it kinetically feasible, the zipper and double helix arrangement would have to be able to coexist in the same strand with easy transitions from one arrangement to the other. While both criteria seem to be satisfied, the transition to a zipper structure can only occur by removal of right-handed twists from the ends of the DNA double helix. Although the current zipper model proposed alternate right and left-handed screw orientations of the chain of five nucleotides apiece, there seems no reason why multiples of five cannot be used, or sections employing different multiples of about five interspersed with classical double helix. Thus there is room for speculation that, while chromosomal DNA may be always double-helical, short sections of DNA in solution may show dynamic fluctuations to regions of zipper form, and greatly modify certain solution properties such as the kinetics of unwinding of short DNA *in vitro* (J. P. Day, personal communication). The important point is that all such conjectures can only be quantitatively justified, in the absence of conclusive experimental data, by theoretical tools related to molecular dynamics. The zipper-double-helix controversy, providing it is not debased by confining it only to the chromosomal DNA *in vivo*, is specifically a problem in molecular dynamics, and the machinery developed by workers like McCammon, Gelin and Karplus is available for resolving it. □

Hepatitis B vaccines

from Arie J. Zuckerman

VIRAL hepatitis has now been identified as a major public health problem occurring endemically in all parts of the world. In recent years, progress in the specific diagnosis of viral hepatitis has proceeded to such an extent that it has revealed a new type of hepatitis which is unrelated to hepatitis A (infectious or epidemic jaundice) or hepatitis B (referred to in the past as serum hepatitis). Precise virological criteria for this new form of hepatitis are not yet available.

The importance of hepatitis B is emphasised by its effects on every field of medical practice, the impact that it has on blood transfusion services and its association with chronic liver disease including cirrhosis and in some regions of the world with primary liver cancer. In addition, infection with hepatitis B virus, particularly in early life, may be

followed by the persistent carrier state. The carrier state may be associated with liver damage, and the world reservoir of carriers at present is estimated to number about 120 million.

Active immunisation against hepatitis B is needed for groups which are at greater risk of acquiring this infection. These groups include individuals requiring repeated transfusions of blood or blood products, prolonged in-patient treatment, patients who require frequent tissue penetration or need repeated access to the circulation, patients with natural or acquired immune deficiency and patients with malignant diseases. Viral hepatitis is an occupational hazard among health care personnel and the staff of institutions for the mentally retarded. High rates of infection with

hepatitis B occur in drug addicts, homosexuals and prostitutes. Persons working in high endemic areas also suffer an increased risk of infection. Women of childbearing age in areas of the world where the carrier state in that group is excessive, are another segment of the population requiring immunisation in view of the increased risk of transmission of the infection to their offspring. Some authors also include military personnel. Consideration will also have to be given to people living in certain tropical and sub-tropical areas in poor socio-economic conditions and where hepatitis B infection is prevalent.

In view of the repeated failure to grow and passage hepatitis B virus (and indeed hepatitis A virus) serially in tissue culture, it has not been possible to develop a conventional vaccine against hepatitis. Attention has therefore been directed towards the use of other preparations for active immunisation. The foundations for such hepatitis B immunogens were laid by the demonstration of the relative efficacy of diluted serum containing hepatitis B virus heated to 98°C for 1 minute in preventing or modifying the infection in susceptible volunteers (Krugman *et al. J. Amer. med. Ass.* **217**, 41; 1971). However, the use of heated whole serum or plasma is unlikely to be licensed for general use.

Hepatitis B small particle vaccines

Since hepatitis B surface antigen, the essentially protein coat of the virus, leads to the production of protective surface antibody as shown in experimental studies and serological surveys, the possibility of using purified 22-nm spherical surface antigen particles is attractive. Such experimental vaccines have been prepared from the plasma of apparently healthy carriers of this antigen. Human hepatitis B infection has been transmitted successfully to chimpanzees, and although the infection is mild, the biochemical, histological and serological responses in the chimpanzees are very similar to those in man. Sensitive laboratory tests are now available for the several markers of hepatitis B infection and these provide the means of monitoring vaccines. Limited numbers of susceptible chimpanzees have been shown to be protected by 22-nm particle vaccines that had been treated by heat or with formalin. Preliminary small-scale safety tests of such experimental subunit particle vaccines in volunteers are now in progress.

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Last year, an experimental, partially purified subunit hepatitis B particle vaccine was administered to patients and staff of a haemodialysis unit. The study did not include a control group, but a marked difference in the incidence of hepatitis B infection was observed subsequently between the immunised group and a group of patients and staff who had not received the vaccine (Maupas *et al.* *Lancet*, i, 1367; 1976).

Although it is generally accepted that the viral subunit preparations when pure are free of nucleic acid and therefore non-infectious, the fact that the starting material for their preparations is human plasma obtained from persons who are persistent carriers of the surface antigen, which is a marker of hepatitis B virus, means that extreme caution must be exercised to ensure their freedom from all harmful contaminating material (World Health Organisation *Tech. Rep. Ser.*, No. 570; 1975). Some concern has also been expressed over the possible induction of harmful immunological reactions to host components, including pre-existing structures of the liver cells, which may be present in such particulate vaccine preparations (see Zuckerman *Nature* 255, 104; 1975; *Lancet* i, 1396; 1976), but reactions of this type have not been observed with the highly purified experimental vaccines in the small number of chimpanzees and individuals immunised so far. The WHO Expert Committee on Viral Hepatitis has recently proposed certain minimum criteria and guidelines for the preparation of hepatitis B vaccines (World Health Organisation *Tech. Rep. Ser.*, No. 602; 1977).

Hepatitis B polypeptide vaccines

Attempts are also being made to prepare vaccines from the constituent polypeptides of the 22-nm spherical particles of the hepatitis B surface antigen. Vaccines prepared from such polypeptides would have an added margin of safety since they would be even less likely than the 22-nm particle subunit vaccines to contain infectious virus or contaminating host proteins that might lead to untoward autoimmune reactions in some individuals (see *Nature* 246, 445; 1973). The potential use of antigenic polypeptides derived from hepatitis B surface antigen is under investigation in several laboratories. A number of these polypeptides are immunogenic and stimulate antibody formation in experimental animals to the group-specific antigenic determinant (Shih & Gerin *J. Immun.* 115, 634; 1975) and subdeterminants of the surface antigen (Dreesman *et al.* *J. Virol.* 16, 508; 1975; Gold *et al.* *J. Immun.* 117, 1404; 1976). Certain of the separated polypeptides also induced a

cell-mediated immune response in experimental animals. In addition, a low molecular weight polypeptide component elicited a cell-mediated response to normal human serum, suggesting that at least one integral component of the surface antigen may contain or be intimately associated with an antigenic determinant related to a human serum protein (Cabral *et al.* *Infect. Immun.* 12, 564; 1975).

The major problems with polypeptide vaccines at present are difficulties in obtaining sufficient quantities of the peptides in pure form and the relatively poor immunogenicity of the polypeptides when compared with the small particles.

Synthetic hepatitis B vaccines

An extension of the use of polypeptide immunogens is the isolation and structural identification of a small surface antigen peptide. This peptide could conceivably be synthesised, function as a hapten, and, when coupled to a suitable carrier may serve as a vaccine. A step in this direction has now been reported by Peterson and his colleagues (*Proc. natn. Acad. Sci. U.S.A.* 74, 1530; 1977). In order to elucidate the structure of the surface antigen, it is essential, of course, to identify its proteins and other components, and much effort has been devoted to this work in many laboratories. Peterson *et al.* describe the identification of two major bands of polypeptides, with molecular weights of 22,000 and 28,000 respectively, and other bands with molecular weights of 16,000 and 40,000–90,000. The purified bands from the 22,000 and 28,000 molecular weight bands had essentially identical amino acid composition to each other and to the intact surface antigen. The most notable feature was the large amount of proline and half-cystine and a large percentage of hydrophobic residues. The amino-terminal and carboxy-terminal sequences of amino acids in these two major polypeptides, which accounted for 75% of the total protein, showed that hepatitis B surface antigen with determinants *adw* consists of a single major polypeptide chain or two homologous polypeptide chains that differ only in limited areas of their structure. The difference in the molecular weight of these two components is due to the carbohydrate moiety of the glycoprotein of the second band. Injection of the 22,000 molecular weight polypeptide emulsified in Freund's complete adjuvant elicited an antibody response in guinea pigs. Antibodies were produced to the group specific determinant and to one of the major subdeterminants of the surface antigen. The 28,000 molecular weight polypeptide, however, did not induce an antibody response and it is suggested

that the discrepancy in the immunogenicity of the glycosylated peptides reported by Dreesman *et al.* and Shih and Gerin (see above) could have been due to contamination of the two major polypeptides.

The momentum towards the production of unconventional hepatitis B vaccines is being maintained, and it would be imprudent to speculate whether successful cultivation of hepatitis B virus in tissue culture, which will lead to the development of ordinary vaccines, will be achieved before a synthetic vaccine appears on the horizon. □

DNA-protein interactions

from Michael Spencer

TWENTY-FOUR years after the unveiling of the Watson-Crick model for DNA it is still unclear how most of its associated proteins interact specifically with it. There are two basic problems: first, how particular DNA base sequences might be recognised by corresponding protein structures, and second whether the protein backbone could adopt a repeating secondary structure to match that of double-stranded DNA. The second of these questions is the subject of a recent paper by Church, Sussman and Kim (*Proc. natn. Acad. Sci. U.S.A.* 74, 1458; 1977).

Their approach has a lineage going back to 1955, when the group led by Wilkins suggested (on X-ray diffraction evidence) that in nucleoprotamine there is an extended polypeptide chain following the narrow groove of the DNA B structure (*Nature* 175, 834). This model was, however, relevant only to a limited class of nucleoproteins, because it relied for its stability on electrostatic interaction between DNA phosphate groups and oppositely charged arginine side-chains. Church *et al.* are interested in whether in other cases, such as the *lac* repressor-operator complex of *Escherichia coli* and the histone-DNA complex, there might be an alternative stabilising mechanism which would "set the stage for the specific recognition to take place".

The new work follows an earlier proposal by Carter and Kraut (*Proc. natn. Acad. Sci. U.S.A.* 71, 283; 1974) that the interacting protein might adopt not a single-chain structure but a two-stranded β -chain helix. These authors

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had been struck by the observation of Chothia (*J. molec. Biol.* **75**, 295; 1973) that β -pleated sheets in proteins often adopt a right-hand twist, because this gives a lower free energy than the planar form. Carter and Kraut discovered that such a twist enabled a β -chain duplex to wind neatly round the outside of a nucleic acid helix. They decided, however, that the model was plausible only for the interaction of proteins with double-stranded RNA (not DNA) in the A form; it was then possible to form hydrogen bonds between the ribose 2'-hydroxyls and the polypeptide carbonyl oxygens.

Carter and Kraut were less concerned with present-day nucleoproteins than with a hypothetical ancestor formed in the prebiotic soup. Church *et al.*, more firmly rooted in modern times after the successes of their group with tRNA structure, persisted in trying to build models that would work for DNA in its biologically relevant B form. In order to match the two-fold symmetry of DNA they restricted themselves to antiparallel pairs of protein chains, but this still left them with two possibilities: the adjacent polynucleotide and polypeptide chains could run either antiparallel (type A) or parallel (type P). Polarities are defined here as running from 5' end to 3' end in a strand of DNA, and from NH₂ terminus to COOH terminus in the protein.

Both models were successfully built, using modern techniques of refinement, to incorporate stabilisation by hydrogen bonds from protein NH groups to the 3'-oxygens of the DNA backbone; this utilised all the free NH groups, half of the total being occupied with holding together the β -chain duplex. To obtain good stereochemistry it was necessary to modify the DNA structure, but in the case of type P the distortions were not serious, the atoms of the sugar-phosphate backbone were moved by no more than about half an Ångström unit. To justify the existence of type A models the authors have to invoke the possible existence of considerably distorted DNA structures, but the only evidence for these comes from some rather controversial work by Bram and Tougaard on fibre diffraction patterns (*Nature new Biol.* **239**, 128; 1972). At least some of the unusual patterns reported are now known to arise only with contaminated DNA (Selsing & Arnott *Nucleic Acids Res.* **3**, 2443; 1976).

Within the β -ribbon alternating side-chains project outwards in the models, so that basic residues could bond to DNA phosphates as in the 1955 proposal. The other alternating sidechains point inwards towards the narrow groove of the DNA, where they could be involved in recognition of the base

sequence. The authors hazard no guesses about how this might be achieved, but they helpfully provide atomic coordinates for aspiring model-builders to try their luck on the problem. Some possible interactions have already been described by Seeman, Rosenberg and Rich (*Proc. natn. Acad. Sci. U.S.A.* **73**, 804; 1976).

The weak feature of all this is, of course, the lack of direct evidence that any such structure exists *in vivo*, or *in vitro* either for that matter. Church *et al.* point out that sequence studies predict β -regions at the DNA-binding end of the *lac* repressor; they also quote evidence supporting the idea that binding occurs in the narrow groove of DNA. What is needed, however, is some crystallographic proof. If current rumours emanating from the eastern counties of England are correct, we may soon have a model of the nucleosome which will shed some light on the matter. A further possibility is that someone will be able to produce fibres of DNA complexed with a pure β -ribbon, rather than with whole proteins containing only limited regions of such a structure. If that happens it will be a cue for old-timers to blow the dust off their fibre-diffraction cameras, and to analyse a new and important structure. □

Sorting out the flu genes

from T. Barrett

THE influenza virus, apart from its importance as a human pathogen, is of great interest to virologists because of some unusual features of its molecular biology. Its surface antigenic determinants—the haemagglutinin and the neuraminidase—are constantly changing, leading to periodic influenza epidemics. When cells are coinfecting with different influenza strains recombinant strains are readily produced. This latter property led to the speculation that its genome was segmented, a fact recently confirmed by several laboratories. The genome consists of eight distinct single-stranded RNA segments (McGeoch *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 3045; 1976; Pons *Virology* **69**, 789, 1976; Ritchey *et al. J. Virol.* **18**, 738; 1976). The virus is negatively stranded, that is, the virion RNA (vRNA) must first be transcribed into complementary RNA (cRNA) before it can be translated into viral proteins.

It was assumed that each vRNA segment represented a single gene since eight virus-coded proteins could be identified in infected cells. These proteins are the three P proteins, thought to have some function in virus transcription and replication, the surface antigens, haemagglutinin and neuraminidase; the matrix protein associated with the virus coat, the nucleoprotein associated with the RNA and finally a non-structural protein which performs some unknown function in infected cells but which is not found associated with the mature virus particle. Both the P proteins and the nucleoprotein are associated with the vRNA in the mature virus particle and can be isolated as a complex which is active in cRNA transcription (Rochovansky *Virology* **73**, 327, 1976).

Preliminary assignment of the gene-protein product relationship was based on the protein size, and the coding capacity of the vRNA segments (Ingls *et al. Virology* **74**, 489, 1976). However, there is room for considerable error in this approach and recent work has focused on getting more definite evidence for the gene-protein product relationship. Comparison of the electrophoretic mobilities of the vRNA segments and the virus-coded proteins of several influenza strains demonstrated significant strain differences, allowing the parental origin of both the vRNA segments and the virus-coded proteins to be determined in recombinants. Palese and his coworkers (for review see Palese *Cell* **10**, 1; 1977) have used these electrophoretic differences to map the gene functions of each RNA segment for two influenza strains—PR8 and HK. Analysis of recombinants which differ from either one of the parental types in one (or few) RNA segments allows one to deduce the gene product of this segment by assignment of the parental origin of the viral proteins. The results obtained with this technique confirmed that the gene order on polyacrylamide gels does not necessarily allow one to assign the protein coding function in order of size. For example in the two strains used in these studies, the fifth RNA segment (in order of electrophoretic mobility) of HK codes for the neuraminidase while in PR8 this segment codes for the nucleoprotein. In both strains the slowest moving RNA segment codes for the third largest protein. The technique, however, is time consuming in that suitable recombinants are required and to repeat the procedure on other influenza strains would require a great deal of effort. Recently a rather elegant and more rapid method of assigning gene functions to particular RNA segments has been developed by a group at Cambridge. Their approach to the problem exploits both the seg-

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mented nature of the genome and its negative strandedness (Inglis, McGeoch & Mahy *Virology* **78**, 522; 1977). Individual mRNA functions can be selectively removed by hybridising total infected cell RNA with isolated individual vRNA segments. The individual vRNA segment will hybridise only with its complementary RNA, which acts as a message for a particular protein, and so make it unavailable for translation in a cell-free protein synthesising system. By analysing the protein products of the cell-free system, the protein for which a particular gene segment codes can be deduced by its reduction or total absence. This novel approach to the problem can be extended to any influenza strain and to other negative strand viruses. Another application which the authors suggest is the identification of gene coding functions of DNA restriction fragments. Since DNA-RNA hybrids are more stable than DNA-DNA hybrids in formamide the technique could be used to fish out mRNAs coded by particular DNA fragments. □

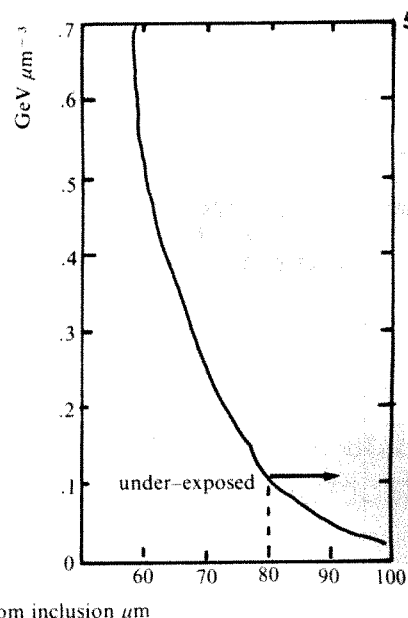
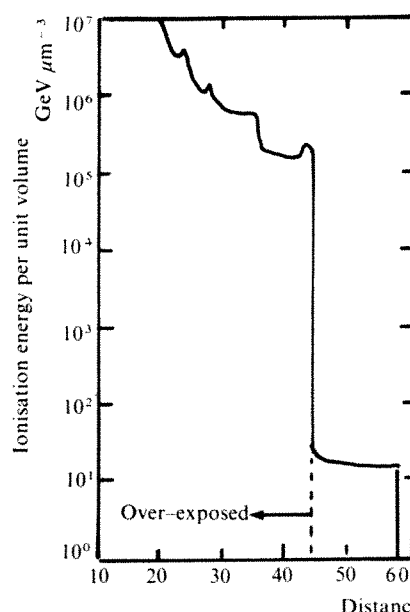
Giant haloes explained

from P. E. Hodgson

A POSSIBLE explanation has recently been proposed for the so-called giant haloes that are sometimes found in mica. The haloes are formed by alpha particles emitted from small inclusions of radioactive minerals inside the mica. These alpha particles ionise most strongly, and therefore cause the most damage, towards the ends of their paths, and so each inclusion is surrounded by a spherical shell of damaged mica, the radius of the shell corresponding approximately to the range of the alpha particles in the mica. In a thin sheet of mica, the region of damage appears as a circle, and the discoloured region of mica is called a pleochroic halo.

Many such haloes have been studied, and most of them have radii that correspond to the energies of the known alpha particles from radioactive minerals. However some haloes were found of much greater radii than these, indicating alpha particles of higher energy than any that are known to be emitted from radioactive minerals. These were called 'giant haloes', and it was conjectured that they might be due to the radioactive decay of superheavy nuclei with nuclear charges much greater than any known nuclei.

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Cumulative alpha and proton ionisation as a function of distance from the centre of the halo. The arrows indicate the critical ionisations corresponding to the inner and outer edges of the halo.

Last year Gentry and colleagues irradiated with 6 MeV protons some inclusions of the mineral monazite that were associated with giant haloes, and found gamma rays were emitted that they associated with superheavy nuclei of charges 116 and 126 (*Phys. Rev. Lett.* **37**, 11; 1976; *Nature* **261**, 627; 1976). Later work, however, showed that these gamma rays can be attributed to the small amounts of cerium often found in this mineral, and extensive studies in many laboratories failed to find any evidence that there was any detectable amount of superheavy nuclei in other specimens of the mineral monazite (*Nature* **265**, 496; 1977).

This work effectively disposed of the claim that superheavy nuclei had been found, but there still remained the puzzle of the origin of the giant haloes. Did these indicate the presence of superheavy nuclei in such small amounts, or with such a short lifetime, that no trace of them remains in the present inclusions, or do they have some other explanation?

An alternative explanation of giant haloes has recently been proposed by Von Wimmersperg and Sellschop of the University of the Witwatersrand in South Africa (*Phys. Rev. Lett.* **38**, 886; 1977). They suggest that the giant haloes are formed when the mica contains some water. The alpha particles emitted from known radioactive minerals then collide with the protons in the water and knock them onwards. Since the rate of ionisation and hence the rate of energy loss is proportional to the square of the charge, protons have a much greater range than alpha particles of the same energy and so the knocked-on protons have on the average a much greater range than the alpha particles, and thus produce

haloes of greater radius.

Such a suggestion can be checked by calculating the radius of a halo that might be expected to be produced by a known radioactive mineral embedded in waterlogged mica. The cross sections for proton-alpha collisions are well known, and so it is quite straightforward to calculate the ionisation, and hence damage to the mica, that can be produced in this way. The calculated curve of ionisation as a function of radial distance from the inclusion is shown in the figure. The outer edge of the halo that would be produced by such a distribution can be estimated from the known threshold ionisation for alpha particles. The inner edge of the halo is determined by a reversal process that sets in above a certain level of ionisation; the mica becomes clear again, probably due to some annealing process. This is necessary for the observation of haloes by the proposed mechanism, since the ionisation goes on increasing towards the centre of the halo.

Assuming that the mass fraction of water in the inclusion is 0.3, Von Wimmersperg and Sellschop calculate that the inner and outer diameters of the haloes produced in this way are about 43 μm and 80 μm , compared with the measured values for giant haloes of 42 μm and 85 μm respectively. These results were obtained from reasonable assumptions relating to the proposed mechanism, without any attempt to adjust them to give an optimum fit. This certainly shows that the proposed mechanism is able to give haloes of approximately the required dimensions, but it still leaves some questions to be answered. It is not mentioned whether there is any evidence for such quantities of water in mica, though of course even if none were found it could always

be maintained that the water had been lost after the giant halo had been produced. It might also be thought that there should be some evidence of the halo produced by the alpha particles themselves, but as shown in the figure this would be removed by the annealing process at high levels of ionisation. $\square$

Muonium hyperfine structure

from Peter Knight

THE muon is a curious particle. It behaves in all respects like a heavy electron, yet there seems to be no explanation of the large ratio of the muon mass to the electron mass. The atom muonium, consisting of a positive muon and an electron, is the simplest system available for precise investigation of the interactions between these so nearly identical particles. Everything we currently know about muonium is completely specified by Quantum Electrodynamics (QED) and the Bethe-Salpeter relativistic two-body equation. Its hyperfine structure is similar to that of hydrogen, but avoids problems of hadron structure so a precision measurement of the muonic hyperfine structure (hfs) checks leptonic QED to high accuracy and could perhaps indicate differences between muons and electrons. V. W. Hughes of Yale University has undertaken a systematic programme of precision experiments on muonium for the past 20 years, and with 11 co-authors has recently reported some greatly improved results on the ground state hfs (Casperson *et al. Phys. Rev. Lett.* **38**, 956; 1977).

Past work on the comparison of experiment with theory for muonium hfs has been limited by uncertainties in fundamental constants such as the fine structure constant α (this was most important), the velocity of light c and the Rydberg constant R_∞ . These constants are now known to higher accuracy. Experimental work was limited by the number of muonium atoms available. The basic principles underlying the latest experiment have remained more or less unchanged from earlier work, and the improvement in accuracy has been made possible by using the higher muon intensity produced by the Los Alamos 'meson factory' LAMPF. Muons are produced by the parity-violating pion decay $\pi^+ \rightarrow \mu^+ + \nu_\mu$ and are spin-polarised with the muon spin direction opposed to the linear momentum. Parity violation not only provides polarised

muons but also provides an elegant monitor of the subsequent evolution of the muon spin. The positive muon decays into a positron and two neutrinos with the positron emitted preferentially along the muon spin direction (and therefore in an opposite direction to the muon momentum). Atomic muonium is formed by charge capture from a krypton gas target at 1.7 and 5.3 atmospheres. The charge capture process does not alter the muonic spin directions so that spin polarised muonium is formed.

In zero magnetic field there are two hyperfine structure levels—a triplet state with total angular momentum $F = 1$ and a singlet state with $F = 0$ separated from the triplet by the hyperfine structure interval $\Delta\nu$, caused by the magnetic interaction between the spin magnetic moments of the electron and the muon. In the strong magnetic field (13.6 kG) used by Caspersen *et al.* the interaction of the magnetic moments with the external field is large compared with $\Delta\nu$. Figure 1 illustrates the magnetic-field dependence and state-labelling of the relevant 1^2S_1 levels (the Breit-Rabi diagram). The relevant good quantum numbers are M_J , the magnetic quantum number for the electron, and M_μ the muon spin magnetic quantum number.

The external magnetic field is in the opposite direction to the muon spin, so $M_\mu = -\frac{1}{2}$ and the states $(\frac{1}{2}, -\frac{1}{2})$ and $(-\frac{1}{2}, -\frac{1}{2})$ labelled respectively 2 and 3 are created in equal proportions. If these muonium atoms are left to decay, the decay positrons are emitted preferentially 'backwards'—opposed both to the muon beam direction and the external field direction. Resonant microwave fields induce transitions at frequencies ν_{12} and ν_{34} between states $2 \rightarrow 1$ and $3 \rightarrow 4$; the muon spin direction changes and the decay positrons are then emitted preferentially in the 'forward' direction of the external field. In other words, resonant transitions are detected by the change in the angular distribution of the decay positrons. Sets of proportional counters and scintillation counters are placed on both sides of the target in a 13.6 kG external magnetic field produced by a precision solenoid.

Caspersen *et al.* have measured ν_{12} and ν_{34} . Measurement of both eliminates the need for a separate NMR measurement of the muon magnetic moment with its associated uncertainties. The combination $(\nu_{12} + \nu_{34})$ is used to determine $\Delta\nu$ and $(\nu_{34} - \nu_{12})$ to determine the muon magnetic moment μ_μ in terms of the proton magnetic moment μ_p . Their results are $\Delta\nu = 4463 \pm 302.35 \pm (52)$ kHz (three or four times more precise than earlier measurements) and $(\mu_\mu/\mu_p) = 3.183 \pm 3403 \pm (44)$. This latter can be used to obtain a new, most precise value for the muon-electron mass ratio $(m_\mu/m_e) = 206.76859 \pm (29)$. The difference between the theoretical value for $\Delta\nu$ (Brodsky &

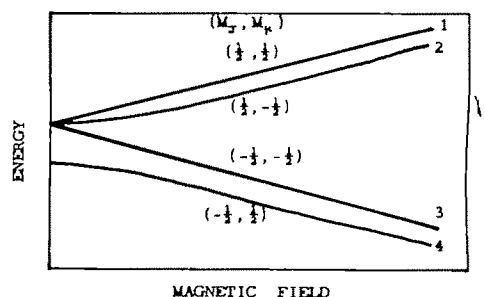


Fig. 1 Schematic diagram of Zeeman splitting of muonium hfs levels. At zero field, energy separation is $\Delta\nu$, the hfs interval.

Drell *A. Rev. nucl. Sci.* **20**, 147; 1970) and the new experimental value is 16.1 ± 6.6 kHz, 2.5 standard deviations of the combined error. The theoretical value is made up of several terms and the discrepancy may be due to an incomplete knowledge of the higher order radiative and recoil corrections to the bound state energy. These calculations are extremely complicated (for example Fulton & Repko *Phys. Rev. D* **14**, 1243; 1976). Weak interaction contributions such as neutral current effects are discounted by Caspersen *et al.* On the other hand, the measured density shifts of ν_{12} and ν_{34} indicate room for further experimental work. $\square$

Biological sun-traps

from J. Coombs

A European Seminar on Biological Solar Energy Conversion Systems was held in Grenoble-Autrains on 9–12 May, 1977. It was organised by Professor D. O. Hall, Kings College, University of London and Dr P. M. Vignais, CNRS, Grenoble, and sponsored by a number of French research agencies.

THE idea of solar energy has risen like the phoenix from the ashes of the world's fossil fuel reserves. The number of publications, reports and research proposals grows steadily, but how realistic are they? Some 300 scientists assembled at Autrans, under overcast skies and persistent drizzle to review the present state of the application of biological solar energy conversion.

The basic tenets remained unquestioned—that solar energy represents the only significant safe, renewable, non-polluting future source

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of energy and chemical feed-stocks. Unlike the advocates of solar energy technology however, biologists suffer from the fact that much of the relevant work is not new, but really photosynthesis, farming and fermentation in another guise. Problems more often relate to application of existing knowledge than to innovation. This is reflected in proposals for the solar energy programme of the European Economic Community in which less than 15% of the expected budget is going to biological projects. Exceptions to this generality, where more basic research is needed, include the possible chemical control of photorespiration, dinitrogen fixation, microbial utilisation of highly lignified cellulose and photobiological production of hydrogen gas.

Reviews of current status of knowledge of photosynthesis (Y. de Kouchkovsky, CNRS, France) and crop systems (P. Chartier, INRA, France) served to define energy flows and metabolic pathways which could give a potential net harvest of about 6% of the 3.8×10^{21} J yr⁻¹ received from the Sun. This contrasts markedly with the total world estimate of 3×10^{21} J yr⁻¹ trapped in photosynthetic products. The energy recovered in useful agricultural produce is several orders of magnitude lower, and there is obvious scope for improvement. However, it was suggested (R. W. F. Hardy, E. I. DuPont de Nemours & Co., USA) that, in the short-term at least, attention to environmental factors, improved varieties, fertilisers and crop protection, coupled with changes in farming objectives—such as a reversal in the decline of high protein grain legume cultivation compared with low protein cereal grain production—will produce the greatest benefits.

With an increasing world population food production must remain the chief aim of agriculture. Plant products to be used for fuel or chemicals must therefore be derived either from agricultural wastes or from new cultural systems—preferably ones which do not encroach on existing agricultural land. Several approaches to this problem were presented.

It is clear that all agricultural systems produce waste, but fortunately not all on the scale described for salad production in France, where studies by J. M. Legarg and A. Buatois, (Université de Lyon) indicated that in some circumstances less than 10% of the produce is eaten. In general, however, reliable estimates of wastes are not available. Detailed surveys carried out by a team from Stanford Research Institute, USA (J. A. Alich *et al.*) in Sutter County, California indicated that (perhaps not surprisingly) manures

tended to be overestimated and forestry residues underestimated by as much as 30%. In this particular county the 16 million tons of low moisture field residues located could provide 2,050 million kilowatt hours of electricity or 13,300 million cubic feet of synthetic natural gas each year. In both cases these figures exceed the present consumption of energy in the given form. This conclusion does not take account of the fact that the bulk of such wastes may be profitably returned to the soil, and that collection may be difficult and costly. In general mill wood and bark residues seem to be most suitable for use as a fuel for process steam or electric power generation. Another important form of waste utilisation is treating straw with ammonia or alkali, a process which doubles the fodder energy value whilst considerably reducing the bulk (M. Israelsen, Biotechnisk Institut, Denmark).

Studies of the technical and economic feasibility of obtaining energy from crops in Australia (H. D. W. Saddler, Australian National University, Canberra), Silvicultural Biomass Plantations (R. E. Inman, The MITRE Corp., Virginia), and marine culture of giant kelp (H. A. Wilcox, Naval Underseas Center, San Diego, California) remain as paper studies, as a result of problems of economics, energy balance and technology. It was therefore refreshing to hear of the commercial production of *Spirulina* (H. Durand-Chastel, Sosa Texcoco, Mexico) and the potential for glycerol production from the alga *Dunaliella* (A. Ben-Amotz, Haifa, Israel), although these were small scale in comparison with the algal ponds used for sewage and waste disposal in the US as described by J. R. Benemann (University of California).

In spite of the lack of large-scale applications for immediate use, optimism was raised by the quality of much of the basic work reported. Here there was a definite emphasis on biological nitrogen fixation. R. A. Dixon (University of Sussex) indicated the possibilities of manipulating genetic information associated with symbiotic organisms, although the possibility of transferring the ability to fix nitrogen to non-legumes remains simply a speculation. The contribution of non-symbiotic nitrogen fixation and loose associations of bacteria with plants remains difficult to estimate. Using a field assay J. Balandreau and Y. Domergues (CNRS, Nancy) found less than 10 kg N ha⁻¹ yr⁻¹ being fixed in both African savannah and around maize plants. The elegant studies of K. E. Eriksson (Swedish Forest Products Research Laboratory, Stockholm) on enzyme mechanisms in-

involved in fungal degradation of lignocellulosic materials could be particularly important since at present no practical process exists for the production of glucose syrups from cellulose, the most abundant carbohydrate.

Hydrolysis, pyrolysis and fermentation of plant products give intermediates which can be converted to most of our present chemical feed-stocks using existing technology (P. Dupuy, INRA, Dijon). The impetus for implementing such technology will come as oil production peaks between 1980 and 1990. Production of ethanol as a fuel by fermentation of raw cane juice is already being investigated in Brazil. More exciting is the possibility of producing hydrogen gas by short-circuiting the photosynthetic process, using reconstituted systems produced by coupling of chloroplasts to hydrogenase as described by D. O. Hall. But both this system and synthetic photo-electric-chemical cells are a long way from practical realisation (A. San Pietro, Indiana).

Although nothing startling emerged from the meeting, a considerable change in the general attitude was noticeable. Proposals are becoming more rational and their limitations are being assessed more critically. The immediate effect may be to reduce waste of both energy and materials. In the longer term it is clear that new avenues of thought are being opened—discussions started during the conference continued unabated as the coaches returned us to the airports—and at last the Sun was shining. □

Magnetism in amorphous alloys

from F. E. Luborsky

An informal meeting on Magnetism in Amorphous Alloys was held on 6 May, 1977 at the University of Sussex, under the sponsorship of the Materials Science Division of the School of Engineering and Applied Sciences. It was organised by Dr A. W. Simpson (University of Sussex) and Professor E. P. Wohlfarth (Imperial College). Participants were invited from the chief European laboratories active in this field.

THERE has been intense research during the past few years on the ferromagnetic behaviour of certain alloys, especially those based on iron and nickel, which can be produced in the form of glassy

tapes by rapidly quenching from the melt. Several of these alloys have attractively high permeabilities and low magnetic losses and there is widespread interest in their possible applications as laminations in transformers and also in smaller electronic devices and in 'bubble memories'. Apart from this technological potential, the purely scientific questions raised by 'amorphous magnetism' have also attracted much attention.

What do we know about the origin of the magnetic properties of metallic alloys that are amorphous? What are the significant unanswered questions related to amorphous magnetism? What is the potential for application of these new transition metal amorphous alloys? These were some of the questions which the Sussex meeting hoped to answer.

In the only formal lecture of the day E. P. Wohlfarth introduced the subject, discussing why interest has arisen in the magnetic properties of the metal-metalloid amorphous alloys, which show soft magnetic properties with applications in transformers, inductors and magnetic sensors. He also discussed what the magnetic properties can tell us about the structure; the reawakened interest in itinerant compared with localised models of magnetism; and finally the origin of the magnetic properties of technological interest.

During the lecture and the discussions which took up the rest of the day many interesting ideas emerged, a few of which are summarised here.

It now seems clear that the non-crystallinity influences the primary magnetic properties in relatively small ways as the result of the variance, or distribution, in the exchange integral. This may be observed directly, for example, in the distribution of hyperfine fields obtained from Mössbauer spectroscopy. However the critical indices of crystalline and amorphous alloys are essentially the same. The relatively large differences in saturation magnetisation and Curie temperature between crystalline and amorphous alloys occurs because of the presence of the metalloid, which is necessary to stabilise the amorphous phase, and not because of the lack of crystalline order.

It was pointed out that very little is known about the effect of preparation variables (quench rate, melt temperature, annealing) on the variance of the exchange integral. The itinerant electron model, as for conventional crystalline alloys, provides a better description of the primary magnetic properties of amorphous alloys than the localised model. There is a smoothing out of the density of states curve for amorphous alloys but since the Fermi level is near the middle of the band, nothing much

will happen to the magnetic moment.

Factors which contribute towards making good soft magnetic materials include primary properties such as moment and Curie temperature, as well as the secondary properties such as the various anisotropies present, magnetostriction and inhomogeneities. The effect of preparation conditions and subsequent mechanical treatment and heat treatment on these secondary properties is particularly important. There are basically three sources of anisotropy: stress-magnetostriction, dipole effects arising for example from inhomogeneities in M_s or from directional atomic pairing, and spin-orbit coupling. The stress-magnetostriction interaction anisotropy is well understood from the many years of work with conventional crystalline alloys. For the amorphous alloys, the stress is understood to develop during the rapid solidification or during subsequent handling, but a fundamental description of magnetostriction and the effect of the non-crystalline structure on magnetostriction are both lacking. The presence of directional atomic order

arising from transition metal atom pairs and metalloid interstitial ordering has been shown to be the dominant source of anisotropy after annealing to minimise or remove the stress-induced magnetostrictive anisotropy. Thus the secondary magnetic properties are controlled by the same kinds of anisotropy as in crystalline alloys, but the magnetocrystalline anisotropy is minimal in a wide range of transition metal-metalloid amorphous compositions. This results in a wide range of compositions, with a range of magnetisations and other properties, of significant technological potential.

Wohlfarth summarised prospects in the near future for transition metal-metalloid amorphous alloys by suggesting that in the next few years there will be more theoretical work on the itinerant and localised models, there will be more relevant measurements on a variety of alloys, and new useful soft magnetic alloys will be developed.

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A hundred years ago

THE *Turkestan Gazette* gives the latest news from M. Prshevsky, dated from Lob Nor, February 22. After having reached this lake by the valley of the Lower Tarim, M. Prshevsky advanced 130 miles east of the lake. The survey and the astronomical measurements of latitudes and longitudes he has made give a totally new aspect to the map of the country. The population on the banks of the Tarim and around the Lob Nor is very sparse; the people speak almost the same language as that of Eastern Turkestan. The flora and fauna of the locality are very poor; some vegetation is found only in the Tarim valley, the neighbourhood being a true desert. During February and March M. Prshevsky was to stay in the Lower Tarim, during May at Yuldus, and during June at Kunghes. About the beginning of July he proposes to return to Kuldsha to begin in August his journey to the Tibet.

A RUSSIAN work, by M. Bogolubsky, on Gold and Gold Mining in Russia, is worthy of notice. It contains very interesting information upon that industry in Russia and Siberia. We observe that the area of gold mines occupies in the Russian empire about

2,100,000 square miles, and now yields yearly about 80,000 lbs of gold, in value upwards of 3,000,000 sterling. The total amount of gold produced in Russia since 1752 has been upwards of 2,500,000 lbs.

MUSCULAR contraction, it is known, is always accompanied with electric phenomena; the difference of electric potential between two points of a muscle, undergoes a diminution, which according to Bernstein, proceeds by about 1/100 of a second the contraction of the muscle. This *electric variation* has been observed in various muscles, and in particular on the heart (by Du Bois Reymond and Kühne), and recently M. Marey has represented it graphically by photographing the indications of a Lippman capillary electrometer. We learn from the *Journal de Physique*, that M. De la Roche has tried the experiment on the heart of a living man. Two points of the epidermis of the chest were connected with the poles of a capillary electrometer, by means of electrodes, formed each of a bar of amalgamated zinc with a plug of muslin at its lower end saturated with sulphate of zinc. Held with insulating handles, the bars were applied, one with its plug opposite the point of the heart, under the left nipple, and the other to another point of the chest. The mercurial column was then seen to execute a series of very distinct periodical pulsations synchronous with the pulse; each pulsation even marked the double movement of the heart (of the auricles and ventricles). The amplitude corresponded to about 1/1000 Daniell.

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articles

Dynamics of folded proteins

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The dynamics of a folded globular protein (bovine pancreatic trypsin inhibitor) have been studied by solving the equations of motion for the atoms with an empirical potential energy function. The results provide the magnitude, correlations and decay of fluctuations about the average structure. These suggest that the protein interior is fluid-like in that the local atom motions have a diffusional character.

RESULTS of X-ray crystallography provide a picture of a globular protein in its native conformation as a well defined, densely-packed structure. Other experimental data¹⁻¹⁰ and theoretical considerations¹¹⁻¹³ indicate that there is considerable local motion inside a protein at ordinary temperatures. Moreover, the structural data themselves show that significant residue or subunit displacements have an important role in the activity of proteins (for example, enzyme catalysis¹⁴, haemoglobin cooperativity¹⁵, immunoglobulin action¹⁶). To obtain a more complete understanding of proteins, it is essential to have a detailed knowledge of their dynamics. In spite of the considerable effort directed toward protein folding¹⁷, very little has been done to investigate the motions of a protein in the neighbourhood of its equilibrium configuration. For certain cases in which the displacements along a suitably chosen co-ordinate can be isolated (for example, aromatic side chain rotations in the pancreatic trypsin inhibitor¹⁸, the opening and closing of the active site cleft in lysozyme¹⁹), it has been demonstrated that empirical energy functions can be used to analyse the motion involved. Here we undertake a more general examination of the internal dynamics of a folded globular protein. The approach used is of the molecular dynamics type¹⁹. In such a study the classical equations of motion for all the atoms of an assembly are solved simultaneously for a suitable time period and detailed information is extracted by analysing the resulting atomic trajectories. The full interatomic potential can be used to obtain the forces on the atoms, so that the method is applicable even when the system is highly anharmonic. The molecular dynamics approach has been very successful in revealing structural and dynamical characteristics of fluids¹⁹. As we demonstrate in what follows, molecular dynamics can have a corresponding role for the internal motions of proteins.

Bovine pancreatic trypsin inhibitor (PTI) (see Fig. 1a) was selected for study because of its small size (58 amino acid residues), high stability and accurately determined X-ray structure^{20,21}. Four water molecules, which are strongly bound in the interior, were included in the dynamical simulation. The potential energy was represented by an empirical function¹⁸ composed of a sum of terms associated with bond lengths, bond angles, dihedral angles, hydrogen bonds, and non-bonded (van der Waals and electrostatic) interactions. Hydrogen atoms are not explicitly considered, but are combined with the heavy atoms to which they are bonded by a suitable adjustment of atomic parameters. This use of 'extended atoms' reduces the

number of interactions which must be calculated and also permits larger steps in the trajectory calculation since the high frequency hydrogen vibrations have been eliminated. Integration of the equations of motion was performed by means of the Gear algorithm²² with time steps of 9.78×10^{-16} s. X-ray coordinates²¹ were used for the initial positions and the initial velocities were set equal to zero. After 100 equilibration steps, the stresses in the initial structure had partly relaxed and the system had an internal kinetic energy corresponding to a temperature of 140 K. At this point, all velocities were multiplied by a factor of 1.5 and 250 more equilibration steps were taken. The added kinetic energy ($250.6 \text{ kcal mol}^{-1}$) partitioned itself between kinetic and potential terms during this interval, and an average kinetic temperature of 285 K was reached. The actual simulation consisted of 9,000 additional steps, corresponding to 8.8 ps. Some equilibration of the bond lengths, bond angles and electrostatic interactions continues during the first 3 ps of the simulation, producing a small rise in temperature. Over the whole simulation, the average temperature is 295 K and the total energy is well conserved, changing by only $0.7 \text{ kcal mol}^{-1}$.

In what follows we first describe results concerned with the relation between the time-averaged and X-ray structures and with the magnitudes of structural fluctuations. We then examine the details of the dynamics, the correlation and damping of fluctuations, and some examples of the larger changes that occur. Of primary importance are the conclusions reached concerning the fluid-like nature of the internal motions, which will clearly have to be considered in developing dynamical models for biological processes.

Time-averaged structure and fluctuations

The time-averaged structure obtained in the dynamics run is near the X-ray structure but not identical with it; the root mean square (r.m.s.) deviation of the α carbons is 1.2 Å and that for all the atoms is 1.7 Å. The largest deviations come from the two ends of the molecule, the external loop (residues 25-28) and exposed sidechains. The two parts of the β sheet (residues 18-24, 29-35) and the α helix (residues 47-56) have significantly smaller deviations.

Use of the X-ray structure as the starting configuration corresponds to $0.4 \text{ kcal mol}^{-1}$ of kinetic energy per atom after equilibration (140 K); for the main dynamics run (295 K), the mean kinetic energy per atom is about $0.9 \text{ kcal mol}^{-1}$. Although there is no tendency to unfold, the dynamic development with this kinetic energy permits the molecule to make small rearrangements throughout; a picture of the structure after 3.2 ps is shown in Fig. 1b. Apparently the PTI molecule samples a series of neighbouring conformations, the average of which is not identical with the X-ray structure. It is not clear how much of the difference is a real effect (in the sense that the structure in the crystal is constrained) and how much is due to inaccuracies in the potential function and the absence of solvent. In lysozyme, comparison of the X-ray structures for the triclinic and tetragonal crystal forms^{23,24} shows an r.m.s.

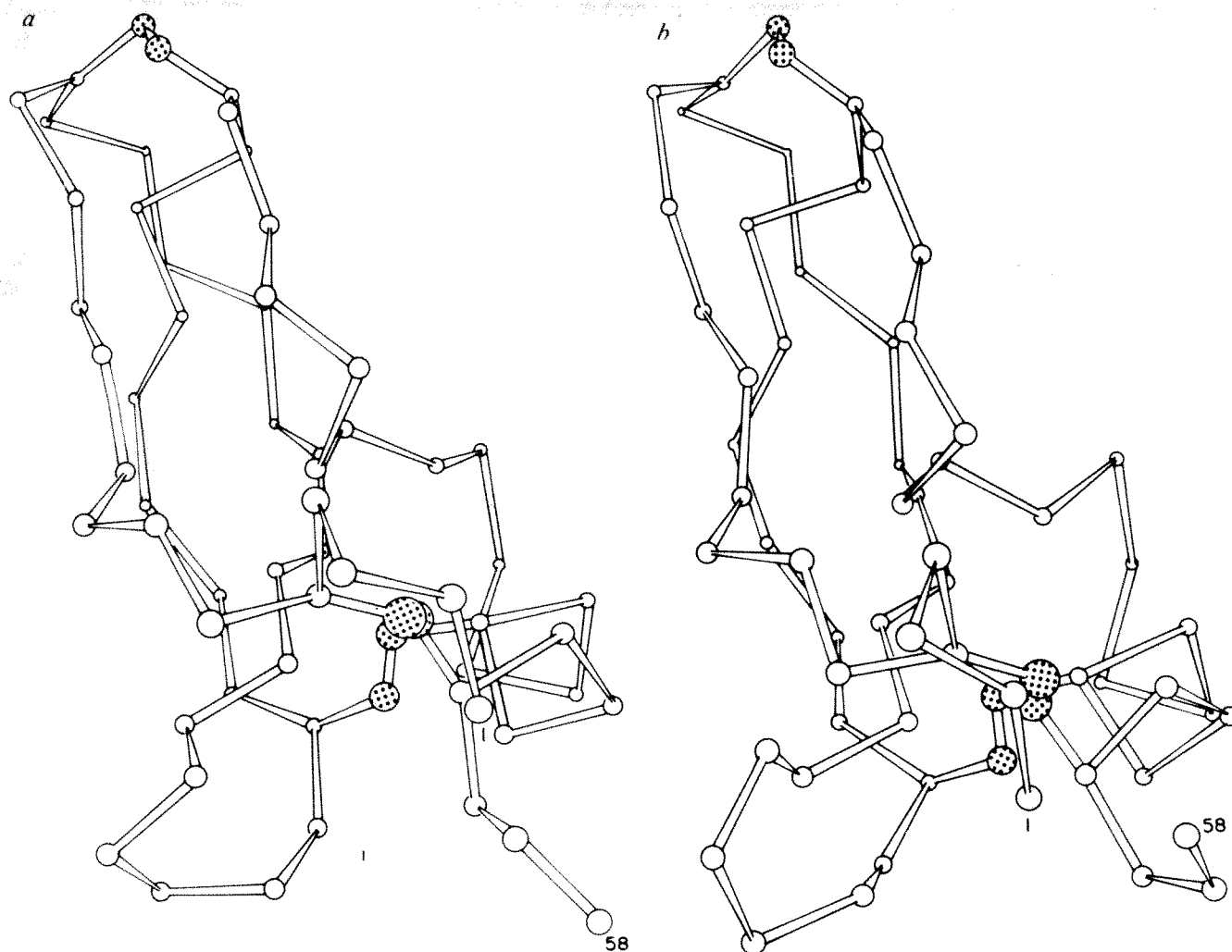


Fig. 1 The peptide backbone (α carbons) and disulphide bonds of PTI. *a*, X-ray structure²¹. *b*, Time evolved structure after 3.2 ps of dynamical simulation.

deviation of backbone atoms equal to 0.5 Å, with the largest changes at the surface of the protein. Also, a variety of other studies^{25–27} support the picture of a protein accommodating its structure to different environments. In any case, the X-ray and dynamic average structures are sufficiently similar that the present results should give a meaningful approximation to the short-time dynamics of PTI.

Figure 2 shows the r.m.s. displacements of the α carbons, relative to the dynamical average structure. The three residues at the carboxyl end of the chain have particularly large fluctuations; these are due to the presence of excess kinetic energy resulting from local strain in the initial structure. Some of this kinetic energy was transferred to the nearby amino end of the chain, which also exhibits relatively large r.m.s. displacements. The important fluctuations in residues 25–28 of the loop connecting the two strands of β sheet seem to reflect an intrinsic softness in this part of the molecule. The smaller fluctuations of the β sheet and α helical regions are also evident. The side-chain atom displacements are somewhat larger than those of the α carbons, but generally show a similar pattern of variation. The average r.m.s. fluctuation of all atoms is 0.9 Å. Particularly large r.m.s. displacements (1.8–3.1 Å) occur in the side-chains of Lys 26, Arg 39, Arg 42, Lys 46 and Met 52; all of these are at the surface of the protein. Overall, the large fluctuation regions correspond to those deviating most from the X-ray structure. In principle, the temperature factors from the X-ray analysis can be compared with the calculated fluctuations. The r.m.s. atomic fluctuation for the α carbons

obtained from the experimental temperature factor is 0.4 Å. Also, some of the more mobile regions correspond to groups of atoms which were not located or had unusually high temperature factors (J. Deisenhofer, personal communication).

The range of variation of the internal coordinates in the static structure can be compared with the dynamical averages and fluctuations. Since the X-ray results for bond lengths and angles correspond to standard values for the different amino acids²¹, we use for comparison the energy-refined geometry (ERG)¹⁸, which reflects the effect of the environment on the coordinates. Selected examples for backbone coordinates are listed in Table 1. It can be seen that the ERG and dynamical averages and r.m.s. variations are in close correspondence. It should also be noted that the average r.m.s. dynamical fluctuation of any given coordinate is two to five times larger than the variation in the coordinate throughout the molecule. The results for the side chains are similar. For the dihedral angle ω , neither the ERG refinement nor the dynamic results show any significant distortions or unusual fluctuations for residues 14–17; this differs from the X-ray results²¹.

The dynamic averages for individual ϕ and ψ dihedral angles differ from the corresponding ERG results by less than 15° for 70 of the 115 backbone angles and by somewhat larger values for the rest (up to 30°). The behaviour of the sidechain dihedral angles is similar to ϕ and ψ .

The r.m.s. dynamic fluctuations of the bond lengths, the bond angles, and the ω angles are essentially constant along the backbone except for slightly (~20%) larger fluctuations in

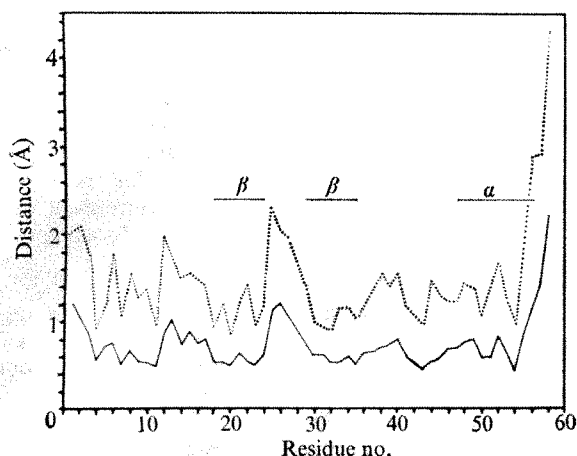


Fig. 2 R.m.s. displacements (—) and maximum displacements (....) of the α carbons, relative to the dynamical average structure.

residues 56–58. For the ϕ and ψ dihedral angles, the r.m.s. dynamic fluctuations range from 10° to 40° and show more variation along the chain; in both the β sheet and α helix regions these fluctuations are about 18° on the average, while in the loop region the fluctuations average about 25° .

Bond lengths calculated from the dynamically averaged set of Cartesian coordinates were uniformly 0.02–0.03 Å shorter than the average bond lengths obtained directly. A similar discrepancy arises in calculating interatomic distances from X-ray diffraction data because of thermal atomic motion²⁸.

Time-dependence of motions

The fact that the individual PTI internal coordinates exhibit dynamic fluctuations of appreciable magnitude raises questions concerning the details of the motions, including their time evolution and spatial correlation.

To illustrate the time-dependence of individual coordinate fluctuations, we present results obtained for the Phe 22. This residue is relatively well buried in the protein and so should exemplify the effect of interactions between different parts of the molecule; other residues examined were found to have qualitatively similar dynamic behaviour. Figure 3a shows the time-dependence of several Phe 22 internal coordinates; the considerable variation in the frequencies, magnitudes of fluctuations, and irregularities of the behaviour of the different coordinates is evident.

To quantitate the average decay characteristics of fluctuations, so as to best analyse the coupling between the coordinate and the rest of the molecule, it is helpful to introduce time

correlation functions²⁹. The time correlation function $C_A(t) = \langle A(\tau+t)A(\tau) \rangle$, for a dynamical variable A is obtained by multiplying the value of $A(\tau)$ by $A(\tau+t)$, the value taken by A after the system has evolved for an additional time t , calculating such products for a representative set of initial times τ and averaging. If A is the fluctuation of a variable from its mean value, $\langle [A(\tau)]^2 \rangle$ is the mean-square fluctuation of the variable for an equilibrated system, while the time correlation function $C_A(t)$ describes the average decay behaviour of the fluctuation. Time correlation functions for the fluctuations of the internal coordinates of Phe 22 shown in Fig. 3a are given in Fig. 3b. Fluctuations of the backbone bond length $N_{22}-C_{22}^{\alpha}$ exhibit a partial loss of correlation in the first 0.1 ps, followed by a very slow decay of the residual small amplitude oscillation. Other backbone and sidechain bond lengths behave similarly. Although the specific oscillation frequencies depend on the force constants and the masses of the bonded atoms, all have long-lived oscillations; among others, the disulphide bond $S_{14}-S_{38}$ was examined and showed no atypical features. The other stiff internal coordinates (bond angles, dihedral angle ω) also exhibit partial loss of correlation in the first 0.1 ps but have persistent though less regular small-amplitude oscillation at longer times.

The relaxation behaviour can be analysed further by obtaining the Fourier transform of the correlation function and finding the dominant frequency components. For the $N_{22}-C_{22}^{\alpha}$ bond, the main peaks are found at 800 cm^{-1} , $1,010\text{ cm}^{-1}$, $1,110\text{ cm}^{-1}$, $1,170\text{ cm}^{-1}$, and $1,430\text{ cm}^{-1}$. These frequencies correspond to the calculated normal modes that involve the largest displacements of the $N-C^{\alpha}$ bond length of the isolated fragment $\text{CH}_3\text{-CO-Phe-NH-CH}_3$ ($787; 952; 1,099; 1,161$; and $1,425\text{ cm}^{-1}$). In addition, there are relatively small contributions from many low frequency motions. Thus, the $N_{22}-C_{22}^{\alpha}$ bond fluctuations are those expected for a strongly coupled system of local oscillators that is weakly coupled to the rest of the protein molecule. For the bond angles and the dihedral angle ω , there are important frequency contributions in the range $300\text{--}1,200\text{ cm}^{-1}$, which correspond to the fragment vibration frequencies associated with these degrees of freedom. Since frequencies below 300 cm^{-1} are also important, it seems that the bond angle and the dihedral angle ω fluctuations are significantly coupled to the protein matrix. Backbone dihedral angles (ϕ, ψ) are seen in Fig. 3b to exhibit considerable damping, though ϕ_{22} preserves long term correlation in a low frequency oscillation of the order of 2 ps. For the sidechain dihedral angle, χ_{22}^2 , associated with the rotation of the phenyl ring, damping produces a nearly monotonic decay with a relaxation time of 0.2 ps. It is clear from the decay behaviour of the correlation functions that the fluctuations of the ϕ, ψ and χ dihedral angles are dominated by interactions with the protein matrix. It is reasonable that torsional motions which involve substantial displacements of large groups inside proteins

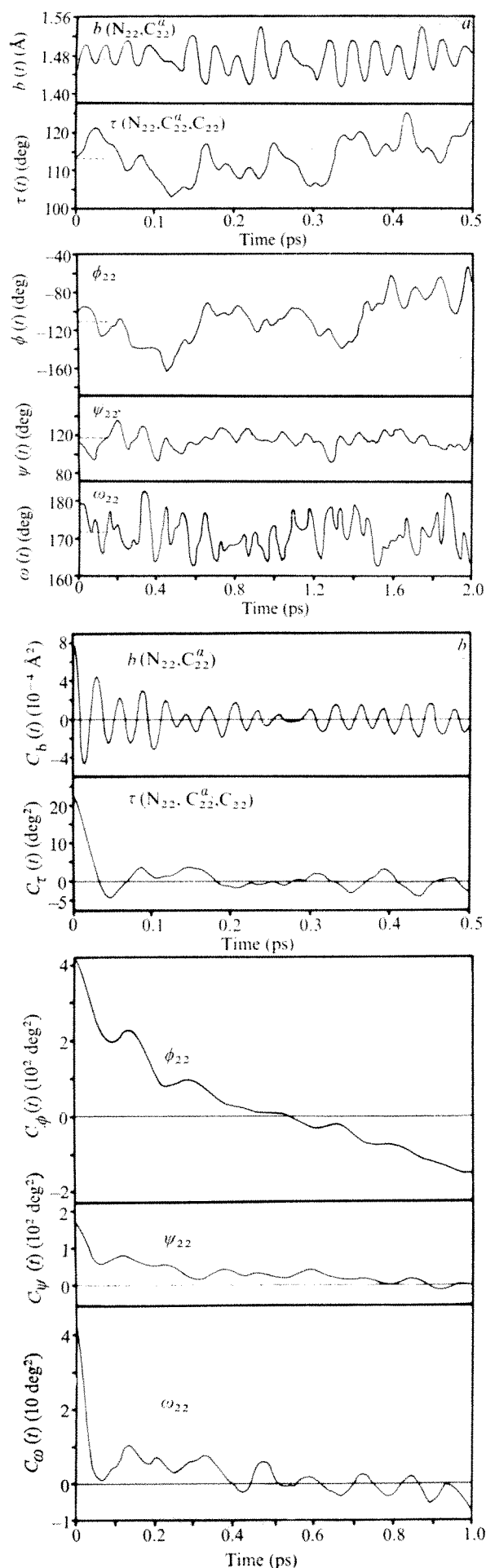
Table 1 Backbone internal coordinates*

Internal coordinate	Average†		r.m.s. variation†		Average dynamical r.m.s. fluctuation‡
	ERG	Dynamics	ERG	Dynamics	
Bond lengths					
N-C ^α	1.476	1.478	0.005	0.004	0.028
C ^α -C	1.531	1.533	0.004	0.004	0.027
C-O	1.237	1.237	0.002	0.003	0.015
C-N	1.321	1.323	0.003	0.003	0.018
Bond angles					
C-N-C ^α	124.6	125.8	1.6	1.7	4.8
N-C ^α -C	113.8	114.9	2.9	2.4	4.8
C ^α -C-N	115.4	114.6	1.8	1.7	4.6
C ^α -C-O	119.7	118.7	1.5	1.5	4.6
Dihedral-angle					
ω	177.9	177.8	5.8	4.7	8.5

* Units are Å for bond lengths, degrees for bond and dihedral angles.

† These values represent the average and r.m.s. variation over the 58 amino acids of a given type of coordinate.

‡ These values represent the r.m.s. dynamical fluctuation of the individual coordinate averaged over the 58 amino acids.



(for example, aromatic sidechains) will have a collective, diffusion-like behaviour, while those subject to smaller steric interactions will retain more of a local character.

The relaxation of individual atom fluctuations seems to be dominated by rotations of the dihedral angles. Atom displacement time correlation functions exhibit qualitative features and characteristic times similar to those shown for the dihedral angles. In many cases, the correlation functions show nearly monotonic decay with relaxation times of the order of a ps.

Correlated fluctuations

The large fluctuations found in the individual dihedral angles might seem to suggest a degree of structural mobility that is greater than that corresponding to the r.m.s. fluctuations of the individual atoms. In fact, the fluctuations of neighbouring dihedral angles are generally correlated so as to minimise disturbances of the backbone and sidechain atoms. For the typical backbone dihedral angle ϕ_{33} , which is at the centre of a strand of β sheet, the observed equal-time correlations of fluctuations of neighbouring ϕ angles and of neighbouring ψ angles are shown in Fig. 4. The large negative correlation $\langle \Delta\phi_{33}(\tau)\Delta\psi_{32}(\tau) \rangle$ is striking and has as a consequence the conservation of the general direction of the backbone and of the approximate position of the sidechains; the larger the individual dihedral fluctuations, the stronger the anti-correlation $\langle \Delta\phi_i(\tau)\Delta\psi_{i-1}(\tau) \rangle$ is found to be. The correlation pattern seen in Fig. 4 is similar to that obtained for an isolated harmonic α helix³⁰. A corresponding pattern of positive and negative dihedral angle correlations is likely to occur generally in strongly conserved regions of secondary structure. The anti-correlation $\langle \Delta\phi_i(\tau)\Delta\psi_{i-1}(\tau) \rangle$ is, in fact, present in other parts of the protein, though somewhat weaker outside the β sheet and α helical regions.

Structure-conserving correlations between dihedral angles also exist in the sidechains. For the eight aromatic sidechains with dihedral angles χ^1 and χ^2 , the normalised correlations $\langle \Delta\chi^1\Delta\chi^2 \rangle / \langle (\Delta\chi^1)^2 \rangle^{1/2} \langle (\Delta\chi^2)^2 \rangle^{1/2}$ range from -0.05 to -0.63; again, dihedral angles with larger r.m.s. fluctuations tend to be more strongly anti-correlated. This correlation is the one required to conserve approximately the orientation of the aromatic rings with respect to the polypeptide backbone.

Concerted motions

In addition to the structure-preserving correlations, a number of concerted atom motions were observed. One of these involves the relatively flexible loop region (residues 25-29) which seems to oscillate as a whole with a period of approximately 6 ps, as well as undergoing changes in shape (see Fig. 1a, b). Some damping of this motion would be expected in solution due to the high solvent accessibility of the loop.

Time correlation functions involving different internal coordinates (for example, $\langle A(\tau+t)B(\tau) \rangle$) can be used to probe the dynamical character of non-local, collective motions in the protein. One such case concerns time correlations between the fluctuations of the dihedral angle ϕ_{33} in the β sheet and the fluctuations of other backbone and sidechain dihedral angles in the molecule. The results show that both strands of the β sheet and some additional residues participate in a low-frequency 'rippling motion' ($\nu = 15 \text{ cm}^{-1}$); within each strand of the β sheet, backbone dihedral angles tend to oscillate with the phase relationships suggested by Fig. 4. Examination of the correlation functions for the atoms involved also shows damped oscillations of the same frequency.

A feature of the dynamics is that certain groups in the molecule undergo transitions from one minimum to another. Such behaviour was found for a number of sidechains;

Fig. 3 a, Time development of selected Phe 22 internal coordinates; the initial time is 4.9 ps after the beginning of the dynamical simulation; average values are indicated by broken line segments. b, Time correlation functions for fluctuations of selected Phe 22 internal coordinates.

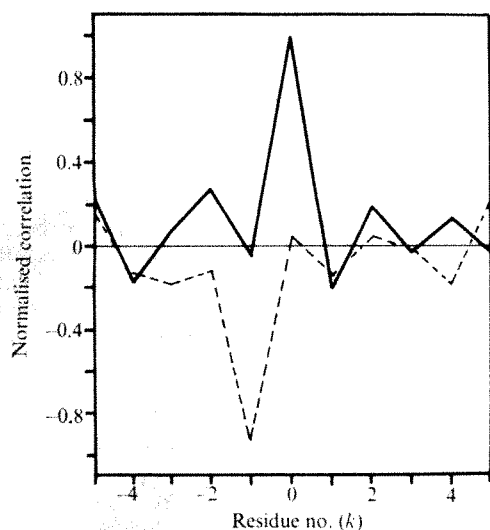
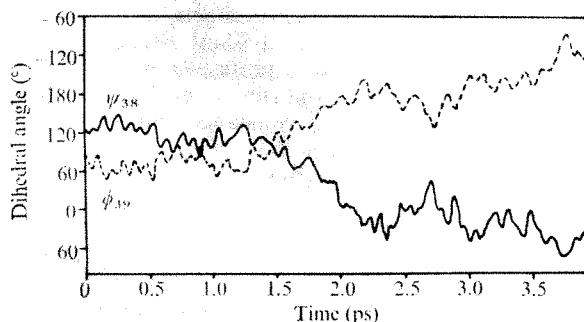


Fig. 4 Normalised equal-time correlations of fluctuations in ϕ_{33} with fluctuations in ϕ_{33+k} (—) and with fluctuations in ψ_{33+k} (---); the quantities plotted are, for example, $\langle \Delta\phi_{33}\Delta\phi_{33+k} \rangle / \langle (\Delta\phi_{33})^2 \rangle^{1/2} \langle (\Delta\phi_{33+k})^2 \rangle^{1/2}$.

for example, Met 52, where χ_{52}^2 moved successively from $-60^\circ \rightarrow +60^\circ \rightarrow -60^\circ \rightarrow 180^\circ \rightarrow 60^\circ \rightarrow 180^\circ$ and Arg 39, where χ_{39}^2 moved successively from $-60^\circ \rightarrow 180^\circ \rightarrow 60^\circ$. The times involved in these rotations vary between 0.2 and 2 ps. Since these sidechains are on the surface of the molecule, they have low barriers due to the protein; their detailed dynamics would be expected to be altered by interactions with the solvent.

In addition to the sidechain reorientations, there occurred a few concerted transitions in the backbone involving ψ_{i-1} , ϕ_i dihedral angle pairs. In these transitions, the anti-correlated angles ψ_{i-1} and ϕ_i rotate in the opposite sense by about 180° so that the intervening amide group flips over, but the backbone and sidechain directions are only slightly altered. The pairs involved are ψ_{38} , ϕ_{39} (near the 14–38 disulphide bond); ψ_{44} , ϕ_{45} ; and ψ_{56} , ϕ_{57} . All of these amide groups are located near the surface of the protein, so that the probability and dynamics of their transitions could be affected by solvent. Nevertheless, it is worthwhile to examine the behaviour since it can serve as a model of structural alterations in the presence of the protein matrix. Figure 5 shows the results for the ψ_{38} , ϕ_{39} pair. The transition is seen to take place in about 1 ps. During this period, the variation in the angles is roughly linear in time and the kinetic energy of the amide group does not show any significant departure from the thermal value. The amide flip is seen to be followed by a conformational adjustment period of 3–4 ps, during which ψ_{38} , ϕ_{39} drift over a relatively small angular interval into their new equilibrium ranges. It is apparent that there is a smooth change from one minimum to the other, while the protein relaxes so that the transition can occur without a large activation energy. The details of the driving torques and other matrix effects remain to be analysed, as do a variety of other larger scale motions that occur.

Fig. 5 Amide group transition; the time development of ψ_{38} and ϕ_{39} during the transition is shown.



Discussion

A molecular dynamics study of the pancreatic trypsin inhibitor has revealed a rich variety of motional phenomena that occur on the atomic level at ordinary temperatures. PTI has been shown to exhibit significant structural fluctuations, and detailed information on the magnitude, correlations and decay of these fluctuations has been obtained.

One important result of the dynamical simulation is that many aspects of the internal motion of PTI suggest that the folded protein is fluid-like at ordinary temperatures³¹. By this we mean that the fluctuations in atom positions, although confined to the neighbourhood of the average structure, have a diffusional character. In other words, the dynamics of atomic displacements are dominated by collisions with neighbouring atoms, at least on the picosecond time scale.

Also of significance is the heat capacity of the protein, which may be calculated from the magnitude of fluctuations in the kinetic energy³². Over the final 2 ps of the simulation, these fluctuations had the average value $\langle (\Delta KE)^2 \rangle = 87 \text{ kcal}^2 \text{ mol}^{-2}$; the corresponding heat capacity per atom is $C = 2.3 k$, where k is Boltzmann's constant. This result, which is midway between those expected for a harmonic solid ($C = 3k$) and for a hard-sphere fluid ($C = 1.5k$) indicates that the potential governing the atomic motions is significantly anharmonic. Similar values have been measured in real fluids; for example, at 84 K in liquid argon, $C = 2.32k$ (ref. 33). The calculated heat capacity of $0.33 \text{ cal g}^{-1} \text{ K}^{-1}$ is consistent with experimental values for proteins¹³.

An idealised model for proteins is that of a dense hard-sphere fluid composed of particles that are connected by flexible links. This implies that many of the dynamical properties (though not necessarily the correct average structure) can be obtained from any potential function which includes the forces that depend strongly on distance (covalent and hydrogen bonds, non-bonded repulsions) and provides sufficient attractive interactions to preserve the compact structure of the native system. Such a representation of a protein has much in common with the hard-sphere model of dense fluids, which has had considerable success recently in reproducing their equilibrium and dynamic properties³⁴. The close correspondence of the protein dynamics with that of the high temperature (300 K) solid phase of normal alkanes is also of interest^{35,36}. The importance of motions in which hard-sphere interactions dominate suggests that in conformational changes the non-bonded contacts will determine the transmission of effects from one part of the molecule to another. One such case of biological interest is the recent determination of the mechanism of tertiary structural change in a haemoglobin subunit, where non-bonded contacts were found to have the dominant role³⁷.

Although the time scale of the simulation is rather short, it provides essential details concerning the dynamics that should be of considerable utility in analysing longer time scale phenomena. A basic result is the r.m.s. atom fluctuation of $0.9 \text{ \AA}$. In a stochastic model that assumes independent atom motions, this provides a means for estimating the probability of larger fluctuations. Of corresponding significance is the nearly monotonic decay of the atom fluctuations with a time constant of $\sim 1 \text{ ps}$. It is also of interest that the kinetic energy of residues 49–58 remains 10–20% above the equipartition value for more than 9 ps; this indicates that vibrational energy can localise for relatively long time periods in proteins; this may be compared with the vibrational relaxation times in polyatomic fluids, which are often of the order of many picoseconds, even when efficient relaxation pathways exist³⁸.

It is clear from the dynamic simulation that the protein with room temperature kinetic energy samples highly anharmonic regions of the potential surface. Further, from an examination of the details of the coordinates during the run, as well as the dynamic average structure, it follows that the protein is able to wander over a range of conformations that are in the neighbourhood of the X-ray structure but not identical with it. The protein

seems to be oscillating in a confined multidimensional space, as shown by the fact that the fluctuations from the average structure are similar during the entire dynamical run. Some of the fluctuations that involve reorientation of sidechains or localised portions of the backbone may represent local minima, though their detailed characteristics have not yet been investigated.

Two limitations of the present model need to be mentioned. First is the approximate nature of the potential energy function. Although in previous applications this function has proved reliable for energy refinement of the PTI structure and the calculation of vibrational spectra of small peptides, some of the details of dynamical simulations of proteins may be sensitive to the choice of potentials. Also, hydrogen atoms have not been explicitly included in the simulation. Their characteristically high frequency motions suggest that replacing them by what can be regarded as an effectively averaged potential may not be a severe approximation; nevertheless, there remains a question as to whether their detailed consideration could introduce somewhat more rigidity into the close-packed structure.

Another limitation of the model is the neglect of solvent. The structure and dynamics of groups at the protein surface are expected to be influenced by hydrogen-bond formation and collisions with solvent molecules, as well as by hydrophobic effects that vary with the exposed area. But, many of the essential features of the internal motions analysed in the dynamic simulation of PTI are not expected to be grossly altered by the presence of solvent. The electrostatic component of the potential energy was found to vary over a smaller range than some other energy components during most of the run, and there was no marked low-frequency alteration of the overall shape of the protein during the simulation. Large variations in these properties, which might be found on a longer time scale, would require consideration of dielectric and hydrodynamic effects, respectively.

It is most important to have available detailed tests of the dynamic simulation. Most closely related are results obtained from fluorescence depolarisation, NMR, fluorescence quenching and hydrogen exchange studies. Measurements of fluorescence depolarisation on a picosecond time scale could be interpreted directly by use of the dynamic results. Other data (for example, fluorescence quenching) require assumptions, such as that of stochastic behaviour, to use the information on fluctuations that has been obtained. For certain rare phenomena that are not represented in the dynamic simulation because they involve high activation barriers (for example, rotation of a tyrosine

sidechain), special methods based on dynamics starting at the transition state may have to be introduced^{39,40}. Comparisons with a variety of experimental data, as well as studies of motional effects on enzyme mechanisms and of conformational changes involved in ligand binding are in progress. A combination of these theoretical approaches with the interpretation of related experiments will provide a unified description of motions in proteins.

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The rings of Uranus: theory

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The structure of the recently discovered rings of Uranus is accounted for by a series of orbital resonances with the satellites Ariel, Titania and Oberon. The observations and their interpretation will considerably improve our knowledge of the mass of the planet and its dynamical oblateness and could influence our ideas on the formation of planets and satellites.

THE recently discovered rings encircling the planet Uranus¹ are the second example we have of a planetary ring system; however, the rings of Saturn and those of Uranus have quite different structures, and different theoretical discussions will be required. In each case resonant interactions, between the particles making up the rings and the perturb-

ing satellites, have to be considered. But in the case of Saturn the rings are wide, and a continuum of orbital periods of the circulating particles exists; the sharply defined orbits for which resonance effects with the satellites can be invoked concern the divisions between the rings. One has to suppose then that a profuse supply of particulate matter was available to populate a disk surrounding the planet, and that orbit-orbit resonances involving particles and satellites have caused certain lanes to be swept clear. In the case of the Uranus ring system the situation is in a sense the opposite: the rings themselves are exceedingly narrow and resonances can therefore not be responsible for defining them by any sweeping action. A set of rings 10 km wide with a radius of the order of 45,000 km must be defined by conditions that lend a stability peculiar to the ring

orbits alone. Resonances with satellites must provide here the explanation for the presence or great concentration of the orbiting material in exceedingly narrow lanes.

An explanation in terms of celestial mechanics of any observed orbit-orbit resonance must have two components: one is the stability of the motion maintaining the precise relationship of periods that is seen; the other is a mechanism for setting up the particular resonance. If only gravitational forces are invoked the system is strictly conservative and the precise condition of a resonance cannot be expected to be reached. Liouville's theorem applies and material cannot become concentrated in the particular domain of phase space represented by a resonance. For this reason other orbital resonances in the Solar System have had explanations involving the operation of slight but significant non-conservative forces, namely the tidal drag between a satellite and its parent planet. It is the secular changes resulting from the drag-forces that drive the system through the various numerical relationships, with the possibility then of being arrested in one of them. The requirement is for the presence of a drag-force small enough so that the slight effects of a resonance can arrest the secular evolution, yet large enough to drive it through some resonance conditions in the time available. It is the lengths of the time-spans that have been available in the solar system that have made it possible for so many systems to be channelled into resonances.

In the case of particles, tidal forces are negligibly small, and another drag force is required. The case that the Poynting-Robertson effect (light-drag) on small particles will feed them into resonant orbits has been discussed before² and seems applicable to the case of Uranus' rings. We thus envisage a supply of small particles having been available in the neighbourhood of Uranus, and the action of the Poynting-Robertson drag (chiefly due to sunlight) having been responsible for driving them gradually, on spiralling orbits, towards the planet. It is understood that such a swarm would settle down, through internal friction, to a common plane, and that this plane would adjust itself to coincide with the equatorial plane of an oblate planet. In the course of this slow spiralling, resonances with the satellites of Uranus will be encountered. At such resonances, when certain precise numerical relations hold between the mean periods of the satellites and of the particles in the disk, it is possible for angular momentum to be transferred between them. A stable condition can then occur when the angular momentum that a particle loses by the Poynting-Robertson effect is just compensated by the angular momentum supplied by the satellites in the resonance.

The search for the appropriate resonances accounting for the rings of Uranus was guided by the following considerations. First, the orbits of the particles slowly spiralling towards Uranus will be closely circular and will lie in the equatorial plane of the planet. The satellites of

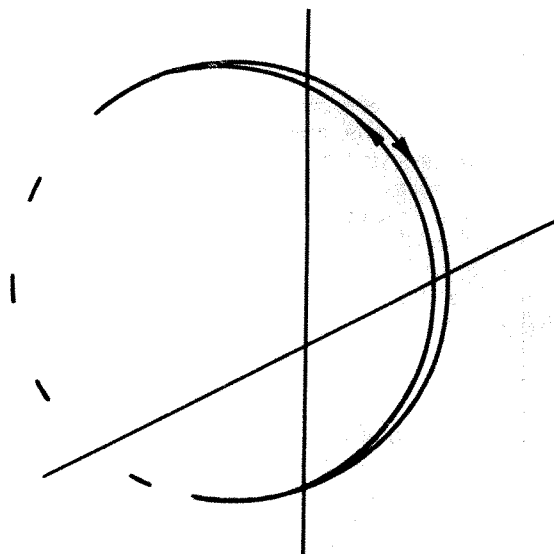


Fig. 1 Libration of the ring particles about a stable longitude is expected, and this would produce a longitudinal dependence of the ring's opacity. The arrows mark the paths of the librating particles in a coordinate frame centred on the planet but rotating with the resonant mean motion. The straight lines represent arbitrary apparent paths of the occulting star projected onto the orbital plane of the particles. The fact that each ring is caught in a different phase at the occultation will account for some being seen on one side of the planet only and some on both.

Uranus also have orbits that are nearly circular and in the equatorial plane (with the exception of the tiny Miranda³). In such circumstances resonance between two bodies has no dynamical significance. This is because in a circular, coplanar system of orbits there are no reference points (apses or nodes), and thus all orbital configurations are equally stable and the system is degenerate. Resonances involving three bodies—a ring particle and two satellites—do not have this degeneracy as one of the satellites can act as a reference point for the other two. In effect, the satellite takes over the role that an apse or a node has in two-body resonance. The precise condition for three-body resonance is

$$qn_1 - (q+p)n_2 + pn_3 = 0 \quad (1)$$

where the n_i are mean motions (suffix 1 refers to the ring particle) and p and q are integers. It follows from equation (1) that in a reference frame rotating with the mean motion of the ring particle, the mean motions of the satellites are commensurate in the ratio $p : (p+q)$.

The strength of such resonances, that is, for our purposes, the amount of angular momentum that can be transferred to the ring particle, is known to depend chiefly on: the order of the resonance, q , (which must be low and preferably unity); the product of the satellite masses; and on a certain function of their orbital radii^{4,5}. From estimates of the function masses based on their visual magnitudes⁶ one would suppose that the combinations of Ariel and Titania and of Ariel and Oberon would dominate (see Table 1). Discounting the tiny Miranda, Ariel is the satellite closest to Uranus.

In comparing the observational data on the rings with predictions based on resonance theory, we consider that the outer two obscuring events, one seen on one side of the planet and one at a different distance on the other, are two separate rings. As the particles in a resonant ring are expected to be librating about a stable longitude (displaced from the longitude of conjunction of the perturbing satellites by an angle dependent on the mean torque that needs to be supplied by the resonance to offset the light drag), the particle distribution in the ring will

Table 1 Products of satellite masses

Satellites	$m_2 m_3$ (Planetary units $\times 10^{-11}$)
Miranda, Ariel	19
Miranda, Umbriel	5
Miranda, Titania	32
Miranda, Oberon	25
Ariel, Umbriel	99
Ariel, Titania	580
Ariel, Oberon	447
Umbriel, Titania	170
Umbriel, Oberon	131
Titania, Oberon	770

have a strong longitudinal dependence (see Fig. 1). In such circumstances, the characteristics of the obscuring events will depend on the phase in which each ring is caught by the occultation. It is only at the time of conjunction of the pair of satellites responsible for a set of rings that these would have a common orientation. Thus, one particular path could produce two narrow events of similar opacity, but a path passing near the centre of libration could produce a broader and more intense event on one side of the planet (possibly even with a double peak, as observed for the outer two rings) and nothing at all on the other. We thus have to account for six rings, whose radii have the distinctive pattern of three pairs.

The two sets of preliminary estimates of the radii of the six rings, as calculated by Elliot *et al.*¹ and Marsden⁷, and the radii of the resonant orbits for the dynamically more significant pairs of satellites, as calculated from equation (1) with $q=1$, are shown in Fig. 2. It is immediately apparent from this figure that the distinctive pattern of the rings is reproduced well by the entire class of first-order resonances that fall in that interval and are associated with Ariel and Oberon and Ariel and Titania—the two pairs of satellites which, chiefly on the basis of the mass products shown in Table 1, we expect to have the greatest dynamical significance.

The systematic differences of several hundreds of kilometres between the two estimates of the ring radii are due to the fact that neither authors^{1,7} have yet made complete allowance for the motion of the Kuiper Airborne Observatory (KAO) and because the offset of the path of the occulting star from the centre of Uranus is imperfectly known. As the separation of the rings is small compared with their radii, however, the errors will be proportional to the radii, and we should expect a plot of one set of estimates against the other to be linear. By the same argument, a plot of each set of observations against the radii predicted by the resonances should also be linear, if indeed the proposed identifications of the resonances are correct. The observed linearity of these plots (see Fig. 3) therefore provides a strong argument in favour of the identifications.

The root mean square deviations from the least squares fits shown in Fig. 3 are 66 km in each case. As Marsden rounded off his estimates of the ring radii to the nearest hundred kilometres we can conclude that the fit of the points to a straight line is as close as could be expected, and thus different from our predictions only by a systematic offset. Elliot *et al.*¹ gave distances of the rings from the centre

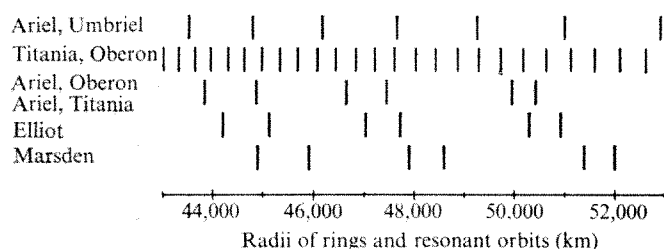


Fig. 2 The two sets of preliminary estimates of the ring radii (labelled Elliot and Marsden) were calculated using the same set of occultation times. The systematic differences of several hundreds of kilometres are due to the fact that neither Elliot *et al.*¹ nor Marsden⁷ have yet made complete allowance for the motion of the Kuiper Airborne Observatory, and because the offset of the path of the occulting star from the centre of Uranus is imperfectly known. Resonant orbits for the dynamically more significant pairs of satellites are shown above the ring radii. The resonant orbits of Ariel and Oberon and of Ariel and Titania are shown on the same line for comparison of their distinctive pattern of three pairs with the observations; from left to right the first in each pair is the Ariel–Oberon and the second the Ariel–Titania resonance; the exact radii are listed in Table 2.

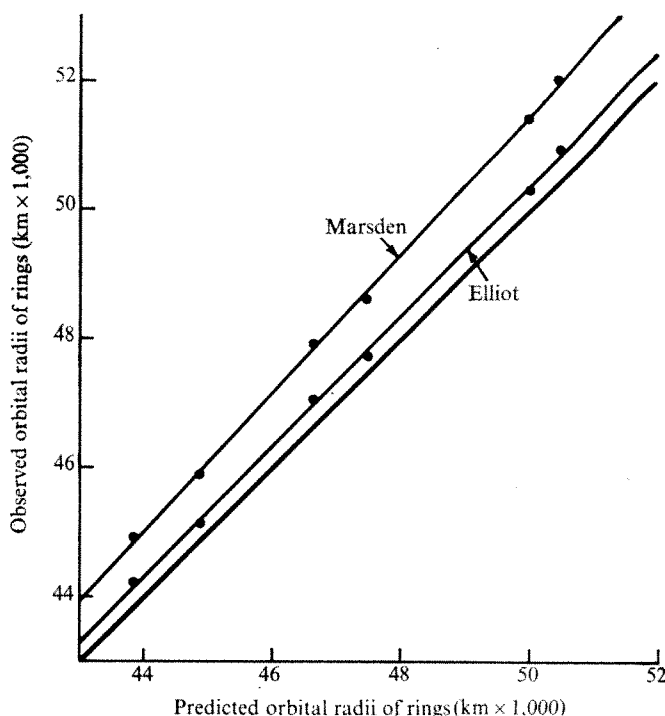


Fig. 3 Preliminary estimates of the radii of the observed rings, as calculated by Elliot *et al.*¹ and Marsden⁷, are plotted against the radii of the resonant orbits of Ariel and Oberon and Ariel and Titania. These radii, and the deviations from the above least squares straight line fits, are also listed in Table 2. A line of slope unity is shown for comparison. The quality of the straight line fit in each case indicates that a very consistent offset rather than a random relation exists between both sets of observational data and the radii predicted by the resonances.

of Uranus before immersion as well as after, and thus from their paper we can obtain two estimates of the ring separations which are independent of the assumed occultation midtime. These are listed in Table 3. The root mean square difference in the separations is 49 km. These differences would seem to be too large to be accounted for by any errors in the data reduction and would therefore indicate that the rings probably have eccentricities and inclinations of the order of 0.001 (these slight departures from exact circularity and coplanarity being generated by the resonant interactions between the ring particles and the perturbing satellites). But, again, we conclude that the straight line fit is as good as could be expected with the present level of observational uncertainty. We therefore conclude that the structure of the rings of Uranus has been determined by three-body resonances with the satellites Ariel, Titania and Oberon.

It seems not to have been noted before that these same three satellites are remarkably close to a first-order resonance condition themselves. In a frame rotating with Ariel, the innermost of the three, the ratio of the relative mean

Table 2 Radii of resonant orbits

Satellites	p	q	Radius* (km)	Deviations (km)	
				Elliot ¹	Marsden ⁷
Ariel, Titania	9	1	50,433	+78	+65
	10	1	47,451	–88	–119
	11	1	44,875	–58	–41
Ariel, Oberon	8	1	49,950	–48	–14
	9	1	46,651	+54	+44
	10	1	43,849	+60	+65

*Assuming $J_2 = 0.012$

Table 3 Distances of rings from centre of Uranus*

Pre-immersion occultations	Ring separation, S	Post-emersion occultations	Ring separation, S'	S-S'
44,144		44,258		
	962		890	+72
45,106		45,148		
	1900		1939	-39
47,006		47,087		
	678		665	+13
47,684		47,752		

*The distances shown correspond with the values shown in Elliot, Dunham and Mink's Figure and were calculated from the times of the obscuring events quoted by Elliot *et al.*¹, assuming: a constant shadow velocity of 11.7 km s^{-1} ; a constant location on Earth; 25,900 km for the radius of Uranus; and an occultation midtime of 21 h 06 min UT.

motions of Titania and Oberon is 0.87414, differing from $7/8$ by one part in 10^3 . This close proximity to a similar resonance ($q = 1$, $p = 7$) as those of the rings suggests that Ariel may have formed from a similar ring, while the rings now remaining are inside or close to Roche's limit. Perhaps other satellites can be found in the locations given by other values of p in the same series.

Positional observations at the distance of Uranus performed with optical telescopes were previously limited to approximately 0.1 arc s, or 1,300 km. The cutting points of the rings and of the disk of Uranus in the occultation can, however, be determined to a much greater accuracy, possibly as good as 3 km (or 2.3×10^{-4} arc s). The identification of the resonances of the rings now allows their periods to be determined with the same precision as those of the satellites. Thus period and radius of orbiting objects will both be known to the new accuracy level, not limited as previously by the telescope resolution. Therefore it will be possible to improve the determination of the mass of Uranus by approximately two orders of magnitude. The knowledge of the planet's radius and its mean density would in any case have been greatly improved by the

occultation observation, but the knowledge of the resonance conditions will further improve these quantities and allow a better value to be obtained also for the dynamical oblateness, J_2 . With many more occultations that will now be useful, Uranus's system of satellites and rings may become the most accurately determined complex dynamical system.

The size distribution of the particles making up a ring of this type is determined by the magnitude of the drag forces and the strength of the resonance. If light-drag is responsible, then the strength of the resonance, which can be calculated by the methods of Greenberg³ and Sinclair⁴, will place a lower bound on the sizes of the trapped particles.

The fact that the Uranus system now provides at least six more examples of the type of three-body resonance described here, raises again the question of the importance that this orbital configuration may have had in the course of the evolution of the Solar System. Dermott (ref. 8 and in preparation) has shown that in the Solar System as a whole, that is, in the planetary system and in the satellite systems of Jupiter, Saturn and Uranus, there is a strong preference for pairs of mean motions, expressed in the rotating system of a third body, to be close to a first-order commensurability. We believe that we can account for the origin of the rings of Uranus by a mechanism of drag forces channelling material into the resonant orbits, as previously discussed by Gold². This suggests that this type of mechanism may have had an important role in the formation processes of the Solar System.

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A consistent description of the nuclear α -decay process

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The essential features of a new theory of α decay and the need for such a theory are reviewed. An approximate relationship between half life and α -particle energy is deduced and checked for the decay of even polonium isotopes. Detailed calculations of one-body widths and spectroscopic factors for the polonium decays are presented and the implications of the results are discussed. It is also shown that the result for the α decay of ^{212}Po is consistent with the reverse α -transfer reaction.

THE process in which a nucleus in its ground or an excited state breaks up spontaneously into an α particle and a residual nucleus is the oldest observed reaction in nuclear physics. Gamow¹ gave a qualitative explanation of this process in terms of the quantum mechanical tunnelling of the α particle through the potential barrier formed by the sum of the real attractive

nuclear interaction between the α particle and the residual nucleus and the Coulomb repulsion between them. Combined with early studies of α -particle scattering from nuclei, this led to the first estimates of the size of the nucleus². Further development of these ideas based on the R-matrix theory of nuclear reactions have made possible satisfactory predictions of relative decay rates³. There has, however, been considerable difficulty and uncertainty in obtaining reliable predictions of absolute decay rates for heavy nuclei. The resolution of this problem has now become important because of the controversy over the possible existence of superheavy nuclei⁴ since the chance of observing these nuclei depends rather critically on their half life for α decay^{5,6}.

Predicting absolute decay rates

The difficulties associated with the prediction of absolute decay rates have two aspects. The nuclear structure part of the problem is concerned with the extent to which the α particle is

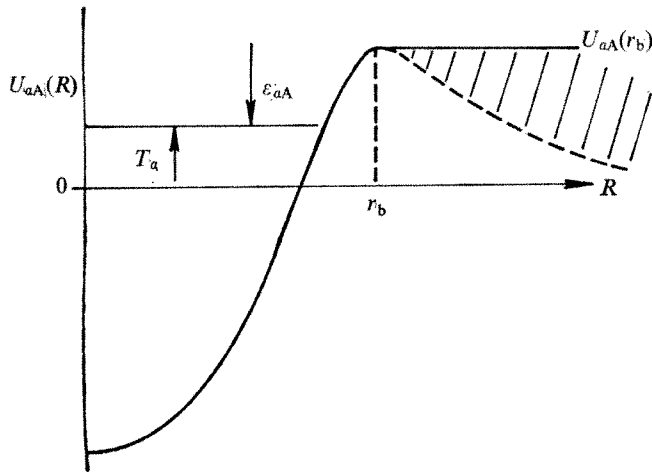


Fig. 1 The shape of the nuclear potential for the α particle in ^{212}Po .

performed in the nucleus; this is measured by the spectroscopic factor S_a which can be expressed as the ratio of the experimentally observed width Γ to the one-body width, that is

$$\Gamma = S_a \Gamma_{ob}, \quad \Gamma = \hbar/\tau \quad (1)$$

where τ is the observed mean lifetime. Within the framework of the shell model it is particularly difficult to show convincingly how the initial shell model state evolves into the final state consisting of the residual nucleus and a free α particle, and in almost all theoretical work using this model the initial and final states are not orthogonal. The nuclear reaction part of the problem is concerned with the emission of the α particle. This is frequently treated by means of the R-matrix theory in which the width Γ of a narrow resonance is proportional to the barrier penetrability P , which in turn depends on the channel radius R_c . The theory does not determine the value of this radius beyond the rather general requirement that it should be sufficiently large to exclude coupling between the various exit channels, and it is well known that a relatively small change in this radius can change the penetrability by an order of magnitude⁷. An approximate method of calculating the penetrability is given by the WKB approximation but this method underestimates the penetrability by at least a factor of five^{8,9}. A further substantial uncertainty in the calculation of the penetrability arises from ambiguities in the phenomenological optical potential which describes the interaction of a free α particle with the residual nucleus^{7,8}.

We have developed a theory of α decay which eliminates all arbitrary radii and ambiguous potentials and provides a consistent description of the initial and final states. It yields spectroscopic factors in agreement with other data but in marked disagreement with the predictions of the shell model. Other authors have attempted such an approach; in one case¹⁰ the arbitrary radius was eliminated but not the potential ambiguities which introduced variations as large as 10^3 in the calculated widths, while more recent work¹¹ removed much of the uncertainty in the potential barrier but retained an arbitrary radius and introduced new uncertainties arising from limited knowledge of the absorptive part of the potential.

The first step is to represent the initial state Ψ_i as an eigenstate of an unperturbed Hamiltonian K so that the difference between K and the exact Hamiltonian H is the perturbation which leads to α decay. An expression for the width Γ of the decaying state can then be obtained using time-dependent perturbation theory or using the unified reaction theory of Feshbach. Both methods yield, to first order⁹

$$\Gamma_{ob} = 2\pi \int dp(E_f) |\langle \Psi_f | H - K | \Psi_i \rangle|^2 \quad (2)$$

where Ψ_f represents the final state with energy E_f and $dp(E_f)$ is

the density of states per unit energy interval. The initial and final states are described by the cluster model, instead of the shell model. In the final state the free α particle interacts with the nucleus through an optical potential and this is calculated using forms for the basic α -nucleon interaction and the nuclear matter distribution of the residual nucleus which are in agreement with other experimental data. The height of the real potential barrier and the position r_b of the peak of the barrier are well determined from low energy α -particle scattering and consequently provided an important test of the calculated potential. For the initial system a bound state is artificially created by continuing the potential at a positive constant value beyond r_b , as shown in Fig. 1. The potentials for the initial and final states are identical for $R \leq r_b$ and the corresponding wave functions are orthogonal to a high degree of accuracy. The decay process arises through the perturbation which lowers the constant potential to its correct value, thus restoring the barrier and allowing the α particle to escape.

Polonium to lead decay

Calculations have been carried out for decays of even polonium isotopes to isotopes of lead, for which the lifetimes and α -particle kinetic energies are well known. For the $O^+ \rightarrow O^+$, $L = 0$, ground state to ground state transitions, equation (2) reduces to

$$\Gamma_{ob} = 2\pi \left| \int_{r_b}^{\infty} u_0(R) [U_{aA}(R) - U_{aA}(r_b)] \tilde{u}_0(R) dR \right|^2 \quad (3)$$

where $\tilde{u}_0$, u_0 are respectively the $L = 0$ bound and scattering wave functions of the α particle whose asymptotic forms are

$$\tilde{u}_0(R) \rightarrow e^{-\gamma R}, \quad \gamma^2 = \frac{2\mu}{\hbar^2} |E_{aA}| = \frac{2\mu}{\hbar^2} [U_{aA}(r_b) - T_a] \quad (4)$$

$$u_0(R) \rightarrow \sin(kR - n \log 2kR + \eta_0), \quad k^2 = \frac{2\mu}{\hbar^2} T_a \quad (5)$$

and U_{aA} is the sum of the nuclear and Coulomb interactions between the α particle and the residual nucleus A. Although there is some latitude in the description of the nuclear interaction because the basic α -nucleon interaction is not uniquely determined, this leads to a variation of only 20–40% in the spectroscopic factor, as indicated in Table 1. Thus the removal of the ambiguity in the potential and the arbitrary radius parameter R_c has eliminated most of the uncertainty in the calculation of α -decay rates, and more precise definition of the barrier height by other experiments would reduce this still further⁹.

Before examining the nuclear structure information represented by the spectroscopic factors we deduce the general relationship between lifetimes and α -particle energies predicted by this theory. In order to obtain such a relationship we neglect the nuclear potential beyond r_b so that

$$U_{aA}(R) - U_{aA}(r_b) = 2Ze^2 \left(\frac{1}{R} - \frac{1}{r_b} \right), \quad R \geq r_b \quad (6)$$

Table 1 Calculated values for decay widths and spectroscopic factors of $L = 0$ decays in polonium isotopes.

Isotope	T_a (MeV)	Potential*	Γ_{ob} (MeV)	$S_a = \Gamma_{exp}/\Gamma_{ob}$
^{210}Po	5.30	H	1.30×10^{-27}	0.029
		H'	1.64×10^{-27}	0.023
^{212}Po	8.80	H	1.02×10^{-14}	0.149
		H'	1.46×10^{-14}	0.104
^{214}Po	7.70	H	5.40×10^{-17}	0.053
		H'	6.80×10^{-17}	0.042

*The symbols H and H' represent different nuclear potentials.

The asymptotic form of $u(R)$ given by equation (5) is likely to be appropriate beyond the outer turning point r_t , which for $L = 0$ is given by $r_t = 2Ze^2/T_a$. Between r_b and r_t the WKB approximation can be used to give¹²

$$u^{(1)}(R) \sim A[K(R)]^{-1} \exp\left\{i \int^R K(r) dr\right\} \quad (7)$$

with $iK(r) = \beta(r)$, where

$$\beta^2(r) = \frac{2\mu}{\hbar^2} \left[U_{aA}(r) - T_a \right], \quad \beta^2(r_b) = \gamma^2, \quad \beta^2(r_t) = 0 \quad (8)$$

Since β^2 is slowly varying, we further approximate the scattering wave function to

$$u^{(2)}(R) \sim e^{i\beta(R)R} \quad (9)$$

so that expression (3) becomes

$$\Gamma \propto \left| \int_{r_b}^{r_t} e^{-\gamma R} \left(\frac{2Ze^2}{R} - \frac{2Ze^2}{r_b} \right) e^{i\beta R} dR + \int_{r_t}^{\infty} e^{-\gamma R} \left(\frac{2Ze^2}{R} - \frac{2Ze^2}{r_b} \right) \sin(kR - n \log 2kR + \eta_0) dR \right|^2 \quad (10)$$

Neglecting the second integral and retaining only the leading term of the first integral, we obtain the approximate expression for the half life T_1 ,

$$\log_e T_1 \propto \{A(T_a) + \log_e B(T_a)\} \quad (11)$$

where, with $x = 2Ze^2/r_b$

$$A(T_a) = 2\gamma r_t = \frac{2Ze^2}{T_a} \left(\frac{2\mu x}{\hbar^2} \right)^{1/2} \left(1 - \frac{T_a}{x} \right)^{1/2} \quad (12)$$

Fig. 2 Plot of $\log_{10} T_1$ for the experimental half lives of even polonium isotopes against $A(T_a)$ (dashed line) and against $A(T_a) + \log_e B(T_a)$ (full line). A value of $r_b = 10.8$ fm predicted by potential H has been used in the calculation.

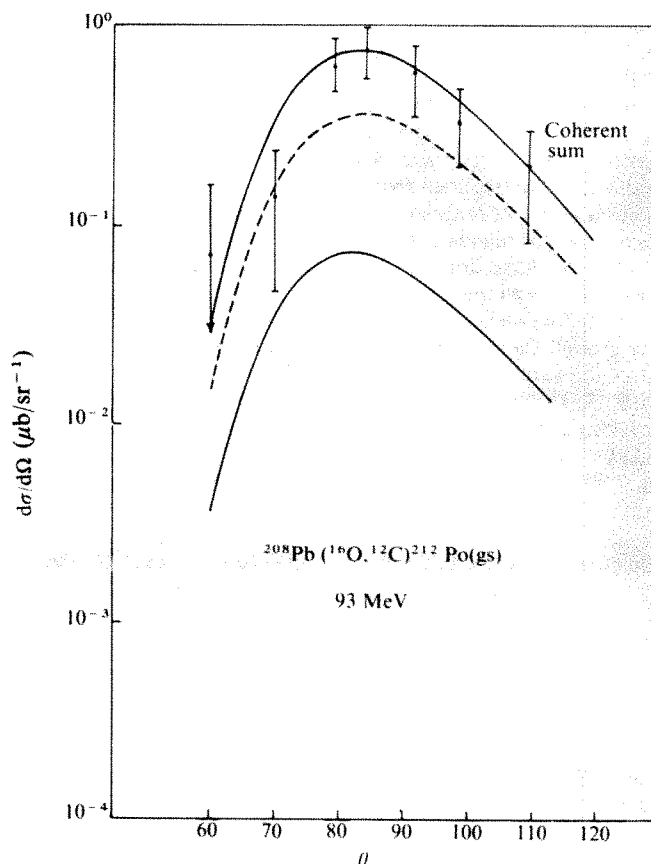
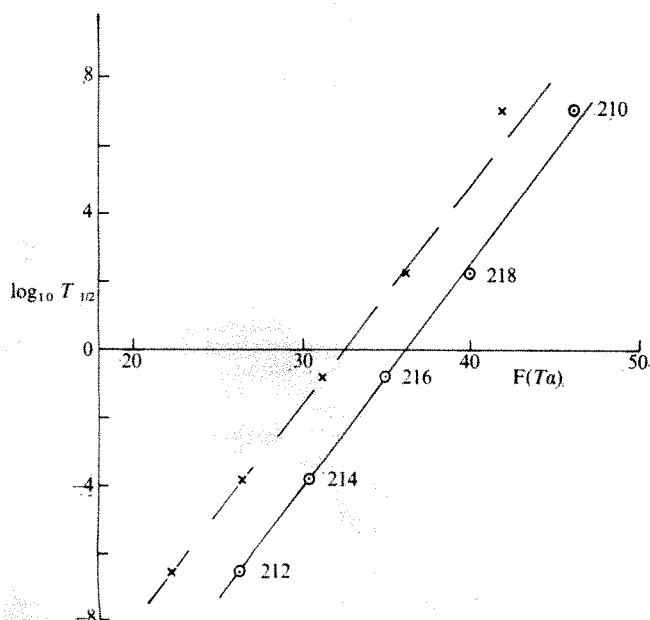


Fig. 3 The angular distribution for the α -transfer reaction $^{208}\text{Pb}(^{16}\text{O}, ^{12}\text{C})^{212}\text{Po}$. The solid line, normalised to the data, represents the coherent sum of the normal DWBA contribution (the dotted line) and an additional contribution arising from our treatment of the α -decaying state (the other solid line).

$$B(T_a) = (\gamma r_b)^2 \left\{ 1 - \frac{r_b}{r_t} \right\}^{-2} = r_b^2 \left(\frac{2\mu x}{\hbar^2} \right) \left(1 - \frac{T_a}{x} \right)^{-1} \quad (13)$$

Although this dependence on T_a does not resemble any of the simple, empirical formulae previously given (see, for example, ref. 13), it can be seen from Fig. 2 that equation (11) does represent the behaviour of the observed half lives of the even isotopes of polonium which cover 14 orders of magnitude, despite the extreme approximations which have been made to derive it. We may reasonably expect that exact calculations using equation (2) should yield the correct lifetime-energy relationship in a wide range of circumstances.

For the particular case of the α decay of the ground state of ^{212}Po to the ground state of ^{208}Pb , which has been widely studied, we obtain spectroscopic factors S_a in the range 0.10–0.15. These values are in excess of the predictions of the shell model (A. Arima and I. Tonozuka, personal communication) by at least two orders of magnitude. It therefore seems that the shell model is inadequate for this nucleus. The most extreme cluster model in which the cluster expansion is reduced to a single term would yield a spectroscopic factor of unity. The small value of S_a obtained for ^{210}Po reflects the need to break the neutron closed shell to remove an α particle, and here the shell model concept is important.

Checking the results

In order to test our results against different data, we have just completed¹⁴ a study of the reaction $^{208}\text{Pb} + ^{16}\text{O} \rightarrow ^{12}\text{C} + ^{212}\text{Po}$ (gs). In this process an α particle is transferred into ^{208}Pb and it is therefore the inverse of the α -decay process. The initial and final states of the heavy nuclei were described in exactly the same way as in the α -decay calculation. The ^{16}O projectile was

described by the model of Kurath¹⁵ which yields a spectroscopic factor of 0.23 for $^{16}\text{O} \rightarrow ^{12}\text{C} + \alpha$. The spectroscopic factor for $^{212}\text{Po} \rightarrow ^{208}\text{Pb} + \alpha$ was then found by making a distorted wave fit to the angular distribution¹⁶ for the transfer reaction. As can be seen from Fig. 3, the fit to the data is good; it yields a spectroscopic factor of 0.14–0.21 which is in good agreement with the value obtained from α decay.

An alternative means of checking the predicted spectroscopic factors is to calculate them directly using the same nuclear model. We have done this for the 18^+ isomeric state in ^{212}Po which decays to the 0^+ (gs), 3^- and 5^- states in ^{208}Pb . Proceeding as before we find that the spectroscopic factor for the decay to the ground state is 0.13 while a simple perturbation calculation gives the parentage of this stage in the cluster model expansion as 0.25. This is satisfactory agreement given the simplicity of the cluster calculation. The ratio of our calculated one-body widths for the decay of ^{212m}Po and ^{212}Po to the ground state of ^{208}Pb is in very close agreement with experiment where, in contrast, shell model calculations using R-matrix theory¹⁷ are greater than experiment by up to a factor of 45, depending on the channel radius. For the transitions to the lowest 3^- and 5^- states we included in our expansion of the initial state only the 0^+ , 3^- and 5^- states of the residual nucleus. The excitation potentials were parametrised directly in terms of the derivatives of the optical potential with the appropriate transition strength taken directly from inelastic α -particle scattering¹⁸. The encouraging results for this calculation, in contrast to the shell model calculations with varying degrees of configuration mixing¹⁷, suggest that what is important in the decay of the isomeric state is not the collective excitation of the residual

nucleus but rather the cluster structure of the decaying state ^{212m}Po .

All the calculations reported here have been restricted to the expected dominant terms in the cluster model expansion but the formalism will allow the study of many additional effects. Our results also suggest that the very complicated many-body structure of the initial state and the formation of the α -particle are more accurately represented by a cluster model expansion. So far, familiar decay processes have been chosen for study in order to eliminate arbitrary parameters, but there is sufficient information on α -particle decay energies and potential energy surfaces available for superheavy nuclei to make possible a much more realistic estimate of α -decay lifetimes in superheavy nuclei.

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letters to nature

Two new D2 QSOs

MOST extragalactic radio sources fall into one of two categories. In one the emission is extended, with two main peaks tens or hundreds of kiloparsecs apart straddling the associated optical object, while in the other the source is compact (typically less than a kiloparsec in size), with optical and radio positions that coincide to within the errors of measurement. More rarely, double sources may belong to a third category, which was classified by Miley¹ as D2, and of which the type example is 3C273. Sources of this class consist of a small variable nuclear component coincident with the optical object, and a second, larger component with a much steeper spectrum. We report here high resolution observations made with radio-link interferometers based on Jodrell Bank which show that the QSOs 3C345 and 3C454.3 have extended radio structure similar to that of 3C273. Hence these sources, previously believed to be compact, should also be classified as D2. Details of the observations are given in Table 1.

Observations at high frequencies^{2,3} with a resolution of a few arc s detect only compact nuclear components in 3C345 and 3C454.3. Our observations show that the nuclear component of 3C345 is unresolved, with an angular size $\theta < 0.1$ arc s, but suggest that the nuclear component of 3C454.3 is extended, with $\theta \sim 0.5$ arc s in position angle $130 \pm 5^\circ$. High resolution VLBI observations⁴ detect fine structure on a scale of about 10^{-3} arc s in both nuclei. The positions of the nuclear components from our observations⁵ agree well with the radio positions by Elsmore and Ryle³,

and by Rogers *et al.*⁶, and with the optical positions of the QSOs⁷.

In addition to the nuclear components, our observations reveal extended emission from a steep-spectrum companion component in each source. The variations with hour angle of the interferometer fringe amplitude for each baseline listed in Table 1 have been fitted by models which assume components with elliptical Gaussian profiles. The component separations are well determined and do not vary with frequency. The sizes of the individual components themselves, however, are less certain. The best fitting model for the structure of each source is shown schematically in Fig. 1, with details given in the legend. The angular separation of the components corresponds to a projected separation at the source of 14 kpc for 3C345, and 39 kpc for 3C454.3, assuming a Friedmann cosmology with $q_0 = +1$ and $H = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$. The corresponding value for 3C273 is 70 kpc.

Table 1 Observations

Frequency (MHz)	Interfero- meter baseline (km)	Minimum lobe size (arc s)	Epoch of observations	
			3C345	3C454.3
408	24	6.3	1969.9 (ref. 16), 1971.7, 1972.7 1971.7	
408	127	1.2	1970.9 (ref. 17)	1974.7, 1975.9
962	24	2.7	1973.9	1973.9
1,666	24	1.6	1973.3	1973.3
1,666	127	0.3	1974.8 (ref. 18)	

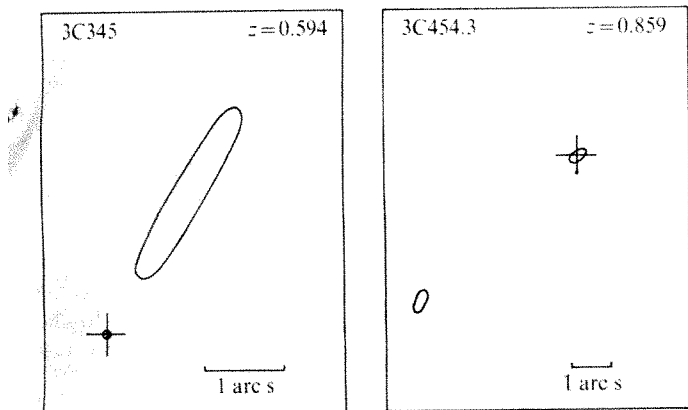


Fig. 1 The radio structures of 3C345 and 3C454.3. North is to the top, increasing Right Ascension to the left. In each source the nuclear component is distinguished by a cross. The components of 3C345 have a separation of 2.1 ± 0.1 arc s in p.a. $149 \pm 6^\circ$. The nuclear component is smaller than 0.1 arc s in all directions; the companion component has a size of $\sim 2.5 \times 0.5$ arc s and is elongated in p.a. $142 \pm 6^\circ$. The components of 3C454.3 have a separation of 5.4 ± 0.1 arc s in p.a. $131 \pm 1^\circ$. The nuclear component has a size of $\sim 0.5 \times < 0.3$ arc s and is elongated in p.a. $130 \pm 5^\circ$; the companion component has a size of $0.7 \times < 0.3$ arc s and is elongated in p.a. $165 \pm 10^\circ$.

Marked variations have been reported in the flux density of 3C454.3 at frequencies below 1 GHz (refs 8, 9). The 408-MHz flux density was 14.0 ± 0.7 Jy at epochs 1971.7 and 1972.7, but had risen to 15.9 ± 0.7 Jy in 1974.7, and was 14.8 ± 0.7 Jy in 1975.9 (flux densities on the KPW scale¹⁰). The fringe amplitude data indicate that it is the nuclear component alone which is responsible for these variations. Our measurements of 3C345 are less extensive but suggest that neither component of this source varied by more than 10% in the 2-yr period of our observations at 408 MHz.

The flux densities of the components of 3C345 and 3C454.3 are shown in Fig. 2 together with the integrated spectrum^{11,12} of each source. Both nuclear components have slightly inverted spectra in the range 408–1,666 MHz, whereas the companion components have steep spectra, with spectral indices -1.4 ± 0.2 and -1.0 ± 0.1 respectively. It is clear from Fig. 2 that the rise in flux density at the low-frequency end of the integrated spectrum may be attributed to the companion component in each case. It may well be that other QSOs with D2 structure could be recognised from the shape of their integrated spectra.

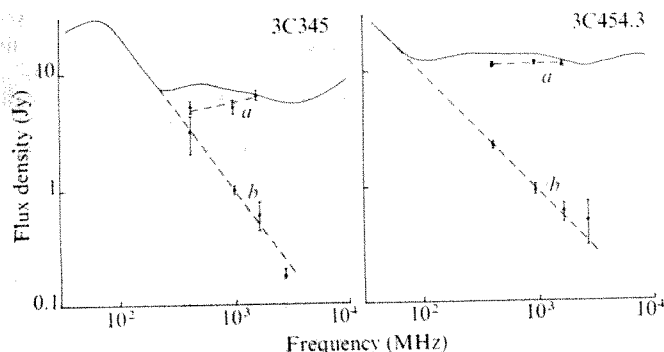


Fig. 2 The radio spectra of 3C345 and 3C454.3. The continuous curves are the integrated spectra from refs 11 and 12. The spectra of the nuclear and companion components are labelled *a* and *b* respectively. The points at 2,695 MHz have been obtained by re-interpreting the visibility data of Bash² in terms of our model structures.

It is interesting to note that 3C273, 345 and 454.3 are all optically violently variable¹³. In the case of 3C273 the companion radio component is associated with a faint optical jet^{14,15}. By analogy, it may be worthwhile to search for optical counterparts to the companion radio components of 3C345 and 3C454.3. There are however considerable practical difficulties in detecting faint optical jets only a few arc s away from ~ 16 mag QSO nuclei.

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Nova shells: matter-bound or radiation-bound?

THE ionisation structure of nova shells is quite uncertain. Many recent papers^{1,2,11} have assumed that the shell is fully ionised during the decline, and that the rate of decline is a sensor of the matter density in the expanding shell. In this letter we propose that the shell of V1500 Cyg is not fully ionised, but rather forms a Strömgren shell. In this case the optical light curve is determined by the decline of the energy production rate of the central object.

The spectrum of a typical fast nova (V1500 Cyg, CP Pup, CP Lac) during outburst is first characterised by a moderately hot ($\sim A$ type) optically thick photosphere. The expanding material becomes optically thin after maximum^{1,11} and after a certain time, which varies from nova to nova, the spectrum changes to that of an H II region or planetary nebula.

Recent papers have assumed that the expanding shell is completely ionised during this nebular phase. In this case the size of the emitting region is determined only by the spatial extent of the shell: the emitting region is said to be matter-bound. The hydrogen emission from a matter-bound envelope is given by

$$E \propto \int N_e N_p a_{ff}(T) dV + \int N_e N_p a_{fb}(T) dV \quad (1)$$

where the electron and proton densities are particles cm^{-3} and the temperature sensitive free-free and free-bound cross sections have the usual definition. We have neglected the effects of line optical depth.

If the shell is homogeneous and hydrogen is the dominant electron donor then equation (1) simplifies to

$$E \propto N_e^2 V \quad (2)$$

For a completely ionised nebula the electron density is directly proportional to the local matter density so we can write

$$E \propto \rho^2 V \propto V^{-2} V \propto V^{-1} \propto t^{-\beta}$$

where t is time. The expansion law is determined by β ; $\beta = 2$ for a thin shell expansion (linear thickness of the shell is independent of time), and $\beta = 3$ for a Hubble law expansion ($\delta r/r = \text{constant}$). The latter may be more appropriate for an explosion. We see that the emission from a matter-bound shell is determined mainly by the matter density. Actually there is also a temperature dependence because of the temperature-sensitive cross sections.

The time of onset of the expansion of a matter-bound shell may be determined from the light curve². The times of onset derived with this technique are typically about one month before optical maximum.

The size of the photosphere at maximum is a simple test of the plausibility of this model. Using V1500 Cyg as an example and assuming that the main shell was ejected 35 days before maximum² at a velocity of 1,500 km s⁻¹ (ref. 3) we find that the optically thick photosphere would have a radius of over 30 AU at maximum, about ten times larger than observed¹. This hypothesis is also in substantial disagreement with the observed blackbody expansion curves^{1,4}.

In this letter we question the basic assumption that the shell is matter-bound. Following the work of Pottasch⁵ we propose that the shell is radiation-bound, that is, that the volume of the emitting region is determined by the balance between photoionisation and recombinations. The most direct evidence that the V1500 Cyg shell was not fully ionised is the observation³ of neutral oxygen emission long after maximum. This was observed both as [O I] 6300, 6363 (ref. 6) and O I 8446, 7774 (ref. 9).

We shall consider the ratio of flux in H β to that in [O I] 6300. This ratio was ≈ 10 for much of the decline⁶. We shall predict this ratio for a fully ionised shell in the following manner. The H β emission from an optically thin unit volume is

$$E(\text{H}\beta) = h\nu_{\beta} N_e^2 \alpha_{\text{H}\beta}^{\text{eff}} = h\nu_{\beta} N_e^2 3.1 \times 10^{-14} \text{ cm}^3 \text{ s}^{-1} \quad (3)$$

where we have assumed a temperature of 10⁴ K and an effective case B recombination coefficient⁷. An upper limit to the energy in λ 6300 may be estimated by assuming that the ¹D₂ level is populated as in LTE at 10⁴ K. The emission from the thermalised ¹D₂ level will be

$$\begin{aligned} E(6300) &= h\nu_{6300} N(\text{O I}) g/U(\text{O I}) e^{-h\nu/kT} A(6300) \\ &= h\nu_{6300} N(\text{O I}) 3 \times 10^{-4} \end{aligned} \quad (4)$$

where we have again assumed a temperature of 10⁴ K. The intensity ratio will be

$$\frac{E(\text{H}\beta)}{E(6300)} = \frac{6300}{4861} N_e^2 N(\text{O I})^{-1} 1 \times 10^{-10} \quad (5)$$

We can predict the fractional abundance of O I by assuming that it is coupled to the fractional abundance of H I by charge exchange

$$\frac{N(\text{O I})}{N(\text{O II})} \approx \frac{9}{8} \frac{N(\text{H I})}{N(\text{H II})} = \frac{9}{8} \frac{\xi}{(1-\xi)} \approx \frac{9}{8} \xi \quad (6)$$

where ξ is the fraction of neutral hydrogen and we anticipate our result that hydrogen is highly ionised. An upper limit to

the oxygen emission can be obtained by assuming that all oxygen is in one of these two species.

We can estimate ξ by equating photoionisation with recombinations and assuming steady state. The balance equation at a typical point in the shell is

$$N(\text{H I}) \sigma \frac{Q(\text{H}) e^{-\tau}}{4\pi r^2} = N_e^2 \alpha_{\text{B}}(I) \quad (7)$$

where σ is the target area for photoionisation ($\approx 6 \times 10^{-18} \text{ cm}^2$), $Q(\text{H})$ is the number of ionising photons emitted by the central object, τ is the optical depth to ionising photons (for a matter-bound shell τ is small so we shall take $e^{-\tau} \approx 1$), and r is the radius of the shell. We will evaluate the parameters at a time 30 d after the outburst. Assuming an expansion velocity of 1,500 km s⁻¹ we obtain a radius of $4 \times 10^{14} \text{ cm}$. Unpublished McDonald scans show that H β had a strength of $5 \times 10^{-17} \text{ J s}^{-1} \text{ cm}^{-2}$ which corresponds to $2.6 \times 10^{17} \text{ photon s}^{-1}$ if we assume¹⁰ a colour excess of $E(B-V) = 0 \text{ mag. 50}$ and a distance of 1,800 pc. The assumption that the shell is matter-bound requires that $Q(\text{H})$ be much larger than the photoionisation rate of $2.6 \times 10^{17} \alpha_{\text{B}}/\alpha_{\text{H}\beta}^{\text{eff}} \approx 2.4 \times 10^{18} \text{ s}^{-1}$. Using these parameters we obtain

$$\frac{N(\text{H I})}{N_e^2} = \frac{\alpha_{\text{B}}(T) 4\pi r^2}{\sigma Q(\text{H})} = 6 \times 10^{-14} \text{ cm}^3$$

or

$$\xi = N_e 6 \times 10^{-14} \approx 10^{-5}$$

where we have assumed an electron density of 10⁸ cm⁻³. The ratio of line strengths will be

$$\frac{E(\text{H}\beta)}{E(6300)} = \frac{N(\text{H})}{N(\text{O})} 7.3 \times 10^{-5} N_e \approx 0.02 N_e \approx 10^6$$

where we have included solar abundances⁸. We see that the observed strength of [O I] 6300 is five orders of magnitude stronger than the predicted value for a fully ionised shell. Enhanced oxygen abundances cannot resolve this discrepancy.

The observed strength of O I is easily understood if the shell is radiation-bound. The O I emission originates at the edge of the hydrogen Strömgren sphere where both free electrons and neutral oxygen are abundant.

The presence of an H Strömgren sphere has important consequences. The optical emission from the envelope will be a sensitive indicator of the emission of the central object at frequencies below the H ionisation threshold since the radius of the Strömgren sphere adjusts itself so that the number of photoionisations equals the number of recombinations. Thus we see that published light curves² of nova shells which are optically thin in visible radiation but which are optically thick to ionising photons are valuable indicators of the decay of energy generation of the central object. The cool outer neutral zone may also provide an auspicious site for the dust formation which seems to be a common feature of the nova outburst¹¹.

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Effect of electrojet on the total electron content of the ionosphere over the Indian subcontinent

THE radio beacon method of measuring TEC along a chain of optimally spaced stations near the equatorial anomaly has been used to monitor continuously the latitudinal extent of the anomaly. Comparisons with the magnitude of the electrojet show a clear dependence of anomaly strength upon the electrojet intensity with an approximate two-hour time delay.

Anomalous latitudinal distribution of electron density in the F2 region, often referred to as the equatorial anomaly, was found by Appleton¹. The diurnal development of the equatorial anomaly has been studied by Rastogi². Dunford³ showed that the equatorial anomaly in the topside ionosphere is correlated with the E region current system near the magnetic equator. With the development of the satellite radio beacon technique numerous workers⁴⁻⁹ studied the Appleton anomaly in total electron content (TEC) of the ionosphere with greater spatial resolution, but with poor temporal resolution, as only a few passes per day of the low orbit satellite could be received. Iyer *et al.*¹⁰ showed that the equatorial anomaly in TEC over India is positively correlated with the electrojet strength. The diurnal development of the equatorial anomaly in TEC is presented here for the first time; and, along with magnetometer records which indicate the strength of the equatorial electrojet, the TEC anomaly is shown for three days in May 1976. The equatorial anomaly in TEC was continuously monitored over the Indian subcontinent using radio beacon signals from the geostationary satellite ATS-6, received at many locations in India, namely: Bombay (19.8°N, 69.8°E), Rajkot (20.8°N, 67.7°E), Ahmedabad (21.5°N, 69.4°E), Udaipur (22.9°N, 70.2°E), Jaipur (25.1°N, 71.9°E), and Patiala (28.2°N, 72.1°E). Coordinates given are 350-km intersections along the ray path to the ATS-6 satellite. Details of the stations in the Indian subcontinent measuring TEC using ATS-6 beacon transmissions have been given by Iyer *et al.*¹¹.

The diurnal development of the equatorial anomaly in TEC and its association with electrojet strength are clearly shown in Fig. 1. Three typical cases are chosen: (1) a normal electrojet day, 18 May 1976, $A_p=3$; (2) a counter electrojet day, where the counter electrojet occurred in early afternoon, 12 May 1976, $A_p=7$, and (3) a geomagnetically disturbed day, 3 May 1976, $A_p=94$. For all three days the horizontal component of the magnetic field (H) for an equatorial station, Kodaikanal, dip 3.4°N, are also shown. The magnetic field during daytime is a representative indication of the electrojet strength. In addition TEC plots against latitude are shown for different local times of the day. On a normal electrojet day the magnetic field shows a flat minimum night value (Fig. 1a). After sunrise the electron density in the E region increases and the H field shows a steady increase until around 1100 h, after which it starts decreasing. Such behaviour of the magnetic field is due to the eastward electric field (E) during daytime. Owing to this electric field and the north-south magnetic field at the equator $E \times B$ drifts are produced and the electrons are lifted to higher altitudes. The lifting of electrons takes place up to a few scale heights above the F2 region peak, beyond which they diffuse along the lines of force and are deposited in the latitude regions of about 15° to 20° dip latitude. This process takes approximately

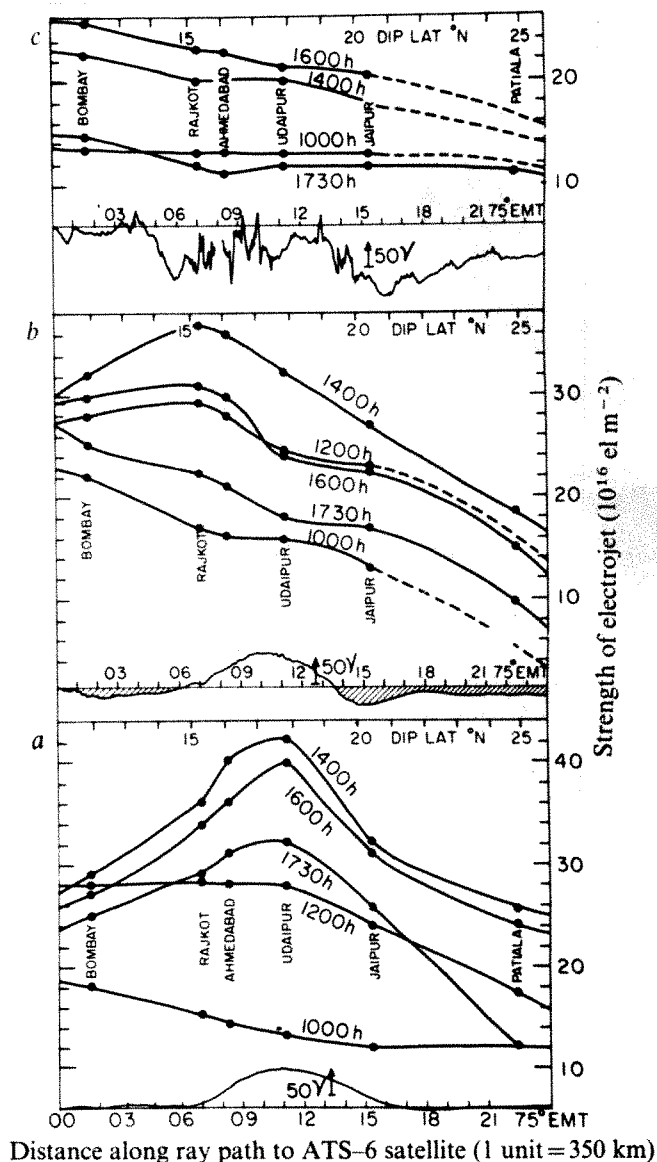


Fig. 1 Diurnal development of equatorial anomaly and strength of electrojet. a, Normal electrojet day. Note that the anomaly is fully developed during afternoon hours. b, Counter-electrojet day, where anomaly is reduced during afternoon hours. c, Disturbed day, where anomaly is not at all developed.

2 h (ref. 10) and any effects in electric fields at E region heights at the equator are seen at latitudes of 15° to 20° after an approximate two-hour delay. At 1000 h, the latitudinal profile of TEC is showing a normal behaviour with high equatorial values (Fig. 1a). As time progresses, at 1200 h, a weak anomaly develops with maximum TEC at Udaipur. By 1400 h more electrons have flowed along field lines to Udaipur and the anomaly is well developed. The process continues until evening and a clear maximum is still seen at 1730 h at Udaipur on this normal electrojet day.

The H component of the equatorial magnetic field shows the start of a normal electrojet in the forenoon hours (Fig. 1b). However, at approximately 1340 h the direction of the H component reverses and a counter electrojet has begun. The latitudinal profiles of TEC for several times on 12 May show a small latitudinal anomaly at 1200 h, reaching a maximum at 1400 h, at the Rajkot ionospheric intersection point (Fig. 1b). But, by 1600 h, and later at 1730 h, there is little, if any, trace of the anomalous latitudinal behaviour in TEC, due to the counter electrojet which developed in early afternoon. The reversal of the

electric field causes downward drift motion at the equator and the movement of ionisation to the latitude range of 15–20° becomes ineffective. The approximate two-hour delay between the start of the counter electrojet and the near-end of the latitudinal TEC anomaly is clearly seen in Fig. 1b.

Data for a highly geomagnetically disturbed day, 3 May 1976, are also shown (Fig. 1c). On this day the electrojet does not occur and the transfer of ionisation from the equator to higher latitudes does not take place to any noticeable extent.

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Hydrothermal plumes in the Galapagos Rift

ALTHOUGH there is indirect evidence that a major fraction of the heat loss from newly-created lithosphere occurs by convection of seawater through the porous crust^{1–3}, it has proved difficult to locate vents of deep-sea hydrothermal systems by direct measurement of the discharge fluid. Local increases in bottom water temperature up to 0.1 °C have been measured by towing arrays of thermistors a few metres above the axes of active oceanic spreading centres^{4,5}, but these data are ambiguous because small temperature anomalies may have a hydrographic explanation. We report here the first conclusive measurements of modified seawater discharging as buoyant hydrothermal plumes from fissures in young oceanic crust. We obtained samples of hydrothermal plumes in the Galapagos Rift⁶, albeit after considerable dilution with surrounding bottom-water, and report the first results of the collection and analysis of these samples.

Our initial estimates of properties useful for detecting deep-ocean hydrothermal fluids were based on the geochemistry of the Red Sea brines^{6,7}, the only oceanic geothermal system previously sampled. In this system the basalt-interaction imprint is unfortunately superimposed on a saturated brine because the seawater flows through evaporite deposits before entering the basalts⁸. Predicted concentrations for constituents masked by the evaporite contribution must therefore be based on laboratory measurements⁹. Our estimates showed that helium isotopes were by far the most sensitive geochemical tracers: a mixture of one part of hydrothermal fluid in ~10⁵ parts of seawater should be detectable in the ³He/⁴He isotope ratio, while a dilution of ~2×10⁴ should be detectable with total helium concentration. The latter figure is comparable with the detection limit for temperature differences, assuming a 50 °C fluid temperature. Transition metals such as Mn and Fe are also highly enriched in the Red Sea brines but the observed enrichments are consistent with an origin from evaporites⁹ and ³He is the only tracer which cannot be derived from such a source⁷.

During the May 1976 Pleiades Expedition of the Scripps Institution, near-bottom hydrographic and geochemical surveys of sites on the East Pacific Rise (4°S, 102°W) and in the Galapagos Rift (1°N, 86°W) were made with a remote sensing and sampling system attached to the deep tow geophysical vehicle of the Scripps Marine Physical Laboratory⁹. The geochemical system included a high-precision CTD instrument to measure conductivity, temperature, and pressure, a light transmissometer, and an array of 8 remotely-triggered 9-litre sampling bottles designed for rapid flushing. The data were telemetered to a shipboard computer which continuously monitored depth, potential temperature (θ), salinity (S), potential density at 4,000 decibars (σ_t), and light transmission in real time. Measurement precision was ± 1 m in depth, ± 0.0015 °C in θ , and ± 0.002 ‰ in S . At each site the instrument package was towed ~8–100 m above bottom at speeds of ~1–2 knots (30–60 m min⁻¹) for about four days, using acoustic transponders for navigation and sonars and cameras for mapping bottom topography.

Near-bottom potential temperature excursions or 'spikes' were encountered in both survey areas; two examples are shown in Fig. 1 (upper traces). Hydrographic or mixing spikes ('false plumes') were characterised as such in the following way. The temperature-salinity (θ - S) relationship for the local water mass was first established by continuous soundings with the vehicle. The observed θ - S relationship (linear at the spreading axis depth) was then programmed into the computer and the appropriate function of θ , S which is invariant to mixing was monitored continuously. Deviations from this relationship were assumed to represent possible hydrothermal plumes and sampling was triggered manually when such deviations were observed.

Fig. 2 shows θ - S data points collected over several hours of towing during the East Pacific Rise survey. Bottom water temperatures west of the rise in this area are ~0.1 °C lower than at comparable depths east of the rise, while at the crest itself there is a transition region which is actually a sharp benthic thermocline (Fig. 2a). Fig. 2b, which contains all the rise-crest data from Fig. 2a, shows that only a single water mass, characterised by a linear θ - S relationship, is present over the entire region. The transition region at the rise crest thus simply reflects linear mixing between the water types on either side of the crest; as the deep tow vehicle navigates through the rough topography the θ record exhibits spikes caused by vertical excursions of the sensor and by encounters with various members of the continuous array of water types available in eddies and streams on the crest. Heated bottom water, however, will deviate from the ambient

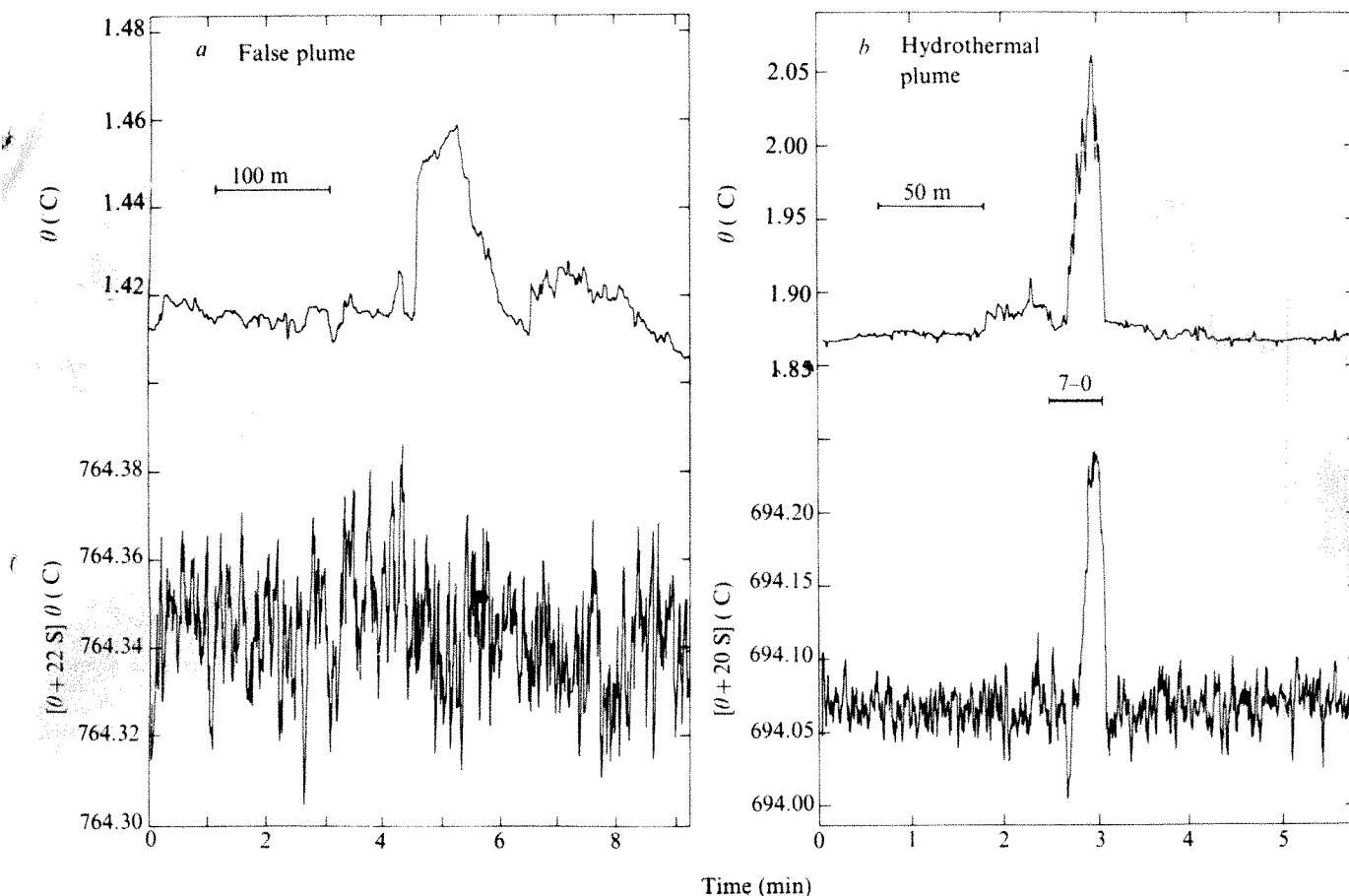


Fig. 1 Temperature records from an arbitrary time zero as the deep tow vehicle is towed ~ 10 m above bottom. Upper traces: potential temperature (θ) plotted at 10 points s^{-1} , corrected for a 200-ms time constant and smoothed by 10-point running average. Lower traces: the θ -salinity function ($\theta + XS$), which is invariant to mixing, with similar correction and smoothing. *a*. A record taken 1.9 km west of the East Pacific Rise axis, shows a 'false plume' in which the temperature spike is not seen in the θ - S function and is thus due to mixing. Tow speed was 50 $m\ min^{-1}$. *b*. The Galapagos Rift site data show a true hydrothermal spike which is reflected in both the temperature and $\theta + XS$ records. Tow speed was 43 $m\ min^{-1}$ and sample 7-0 was collected during the interval shown beneath the θ record.

θ - S relationship characterised by $\theta + XS = \text{constant}$, where X is the negative slope on the θ - S plot (Fig. 2b). Because of the possibility of conductive heating, the detection of such a ' θ - S spike' is a necessary, but not sufficient, con-

dition for the existence of an actual hydrothermal plume.

The lower trace in Fig. 1a is the real time record of the θ - S function measured concurrently with the East Pacific Rise θ record shown above, and plotted to the same

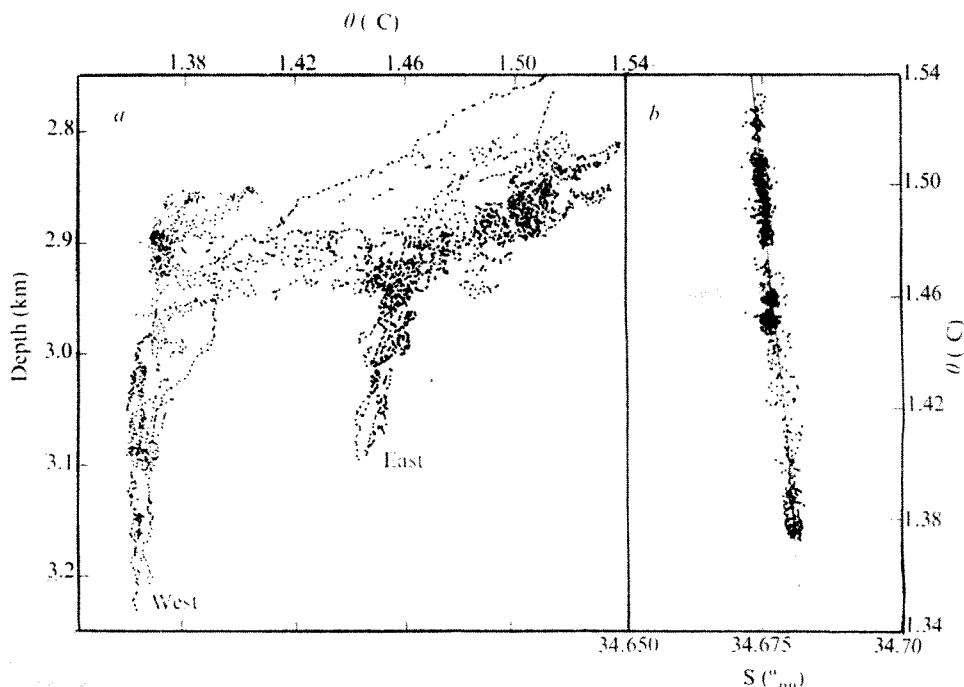


Fig. 2 Near-bottom (10-100 m) potential temperature data points measured over several hours of towing at the East Pacific Rise survey site, plotted against depth (Fig. 2a) and salinity (Fig. 2b). The θ - S diagram (2b) shows that the entire array of water types represents a single water mass; the θ - S slope here ($= -X$) is -22 .

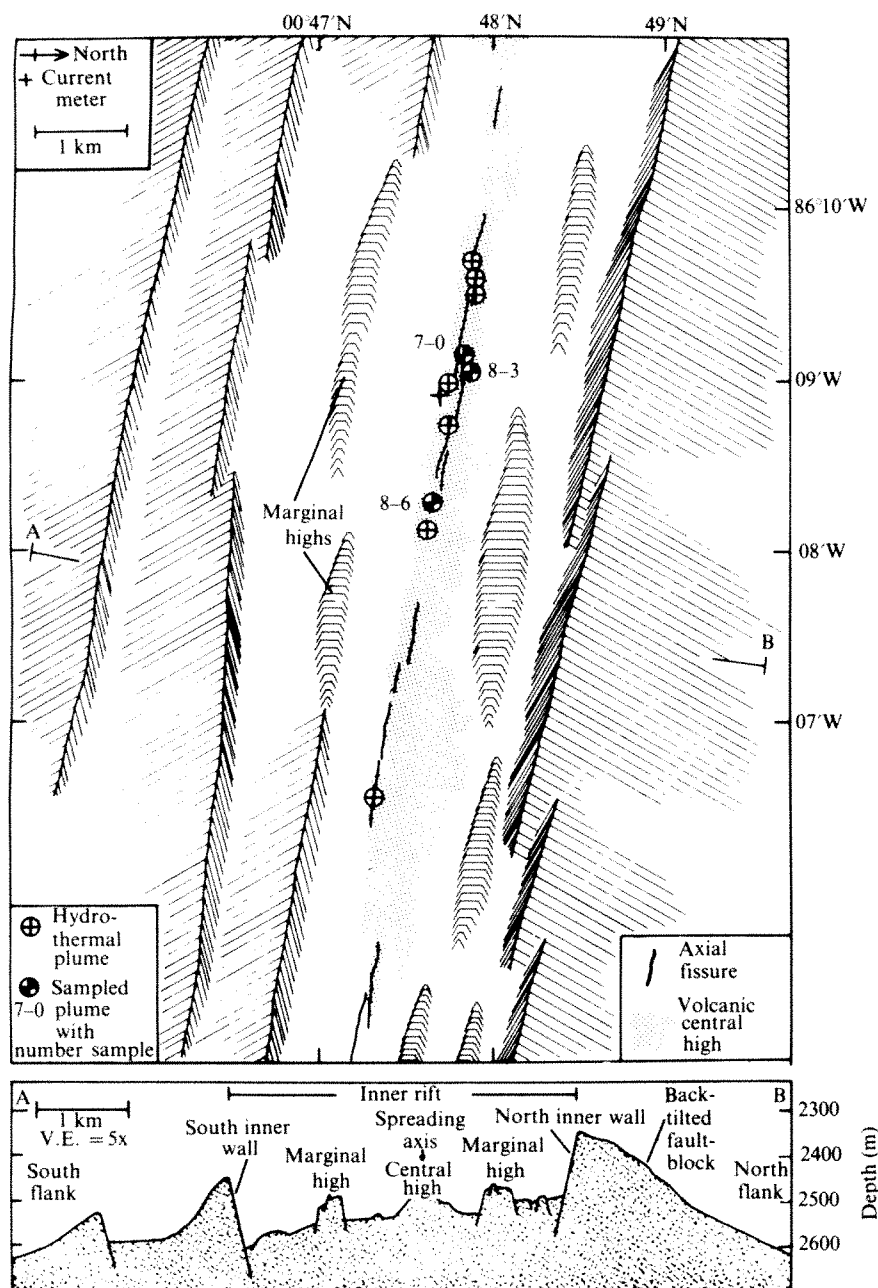


Fig. 3 Locations of hydrothermal plumes observed in the Galapagos Rift, with topography summarised from a deep tow structural map¹⁴. Numbers refer to plumes actually sampled. In addition to the axial fissures shown there are many fissures and minor faults along the minor rift margins; no plumes were detected in these marginal areas.

vertical scale. This spike is clearly due to mixing and is labelled a 'false plume'. It is apparent that a ' θ -S spike' requires an amplitude of $>0.02^\circ$ for detection due to the scatter introduced by the conductivity measurement. Within this limitation, no hydrothermal spikes were observed during the East Pacific Rise survey.

Fig. 1b shows a potential temperature spike measured on the Galapagos Rift which shows a corresponding excursion in the θ -S function. In this area the near-bottom temperature structure reflects mixing between bottom water entering the Panama Basin through the Ecuador Trench¹⁰ and warmer overlying water which spills across the Carnegie Ridge¹¹. The θ -S excursion shows clearly that in this case the θ anomaly is not caused by mixing. Sample 7-0, collected somewhere in the indicated time interval, had a $^3\text{He}/^4\text{He}$ ratio equal to twice the atmospheric ratio, a spectacular anomaly relative to 'background' water which has a ratio only 30% greater than atmospheric¹². This is by far the largest $^3\text{He}/^4\text{He}$ ratio yet observed in deep ocean water and there is thus no

doubt that an actual hydrothermal plume was sampled.

Fig. 3 shows the bathymetry of the Galapagos Rift area as surveyed during the present study. The lava flows in the inner rift valley are fractured by many small faults and fissures along the rift margins, but near the central axis fracturing is confined to a few fissures about two or three metres wide extending along the central high^{13,14}. Ten temperature spikes with corresponding ' θ -S spikes' were observed in this region during the shipboard survey; the locations of these ten spikes are plotted in Fig. 3 which shows that all ten lie on the axial fissure in the centre of the inner rift. Three of these spikes, 7-0 (the largest of the ten spikes) 8-3, and 8-6, were sampled and analysed; all three showed $^3\text{He}/^4\text{He}$ and radon anomalies relative to background water, verifying that the θ -S excursions in these ten locations represent true hydrothermal plumes.

A preliminary analysis of the remaining tow data has shown no evidence of hydrothermal activity off the central axis of the Galapagos Rift. One area of special interest

in our survey was a region of 5–20 m high sediment mounds ~30 km south of the central axis which have been described as possible hydrothermal vents¹². In this area the water structure is so uniform that hydrothermal spikes of ~0.002 °C should be observable, yet even with this sensitivity there was no evidence of hydrothermal plumes associated with the mounds.

Several of the hydrothermal plumes showed a small decrease (~0.1%) in light transmission; filtrates from the three sampled plumes and water have been distributed for a number of geochemical measurements. The helium and radon measurements are described in an accompanying paper¹². Our results show that there is a one-to-one correspondence between the hydrographic identification of samples as heated water and the presence of excess quantities of ³He/⁴He and Rn in these samples. Together, these results constitute the first conclusive identification and sampling of hydrothermally circulating seawater in a deep-ocean spreading centre. Based on these findings, a detailed study of the Galapagos Rift hydrothermal vents using a manned submersible is now in progress.

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Mantle helium in hydrothermal plumes in the Galapagos Rift

THE ³He/⁴He ratio in deep Pacific water is 20–30% higher than in atmospheric helium because of injection of primordial helium from the mantle^{1,2}. The largest ³He enrichments in the Pacific have been found in water on the crest of the East Pacific Rise where the isotopic ratios indicate² that the excess helium component has a ³He/⁴He ratio about ten times the atmospheric ratio, in agreement with the ratios measured in trapped helium in the glassy rims of oceanic tholeiites^{3,4}. Recent measurements in this laboratory⁵ have shown that the hot brines in the axial rift of the Red Sea are very highly enriched in mantle helium. ³He and ⁴He are respectively 3300 and 380 times supersaturated relative to atmospheric solubility equilibrium in seawater, with a ³He/⁴He ratio of 1.2 × 10⁻⁵, or 8.6 times the ratio in atmospheric helium. Comparison of the enrichments of various elements in the Red Sea brines and

in brines associated with salt domes⁶ shows that helium is the only component in the Red Sea brines which unequivocally requires derivation from hydrothermal circulation of seawater in basalts. The helium isotopes are thus an extremely powerful and sensitive tracer for the detection and mapping of hydrothermal systems in oceanic spreading centres.

The Red Sea brines represent an extreme example of the most probable mechanism for the actual injection of mantle helium into the deep sea, namely the penetration and hydrothermal circulation of seawater in basalts on the crests of mid-ocean rises. In the Red Sea the circulating fluid is a brine because the downward penetrating seawater encounters evaporites before reaching the basalt⁶, and thus the upward convecting fluid is stabilised in brine pools in depressions on the sea floor. In normal oceanic spreading centres, however, where evaporites are absent, the heated seawater should emerge as hydrothermal plumes which should be detectable by the association of temperature anomalies with the mantle helium signature. In this letter we report the first ³He measurements on such plume samples, showing that hydrothermal circulation of seawater in basalts is an active process at spreading centres which transfers mantle helium to deep ocean water.

In May 1976 nine samples of bottom water were collected along the central axis of the Galapagos Spreading Center^{7,8} using a newly-devised hydrographic sampling sled^{9,10} attached to the Scripps Institution of Oceanography deep-tow instrument. The location of the samples relative to the local topography is described in the accompanying paper¹⁰. Three of these samples were collected during 'hydrothermal spike' temperature excursions whose origin could not be explained by turbulent mixing within the ambient water mass. The samples were taken about 10 m above the axial fissure opening, at the same level as the CTD sensor which recorded the temperature anomalies. When the sled was recovered, samples for helium analysis were sealed in copper tubing pinch-clamp containers and returned to the laboratory for analysis on a ³He/⁴He ratio mass spectrometer as described previously^{3,5}. Radon activities were measured at sea on six of these samples, using our normal shipboard extraction and counting techniques¹¹ with the exception that the analyses were made on one, rather than twenty, litres of water.

Table 1 lists the ³He results on the three samples taken during temperature spikes (7–0, 8–3, and 8–6) and on the samples of normal or 'background' bottom water where no temperature anomalies were observed on the continuous tow record. The measurements are tabulated as percent deviations of the ³He/⁴He ratio (*R*) from the atmospheric ratio (1.40 × 10⁻⁶), that is

$$\delta(^3\text{He}) = 100 [(R/R_{\text{Atm}}) - 1]$$

The mean background water anomaly is $\delta(^3\text{He}) = 30.4\%$, in excellent agreement with values of 30.3 and 31.0% observed on the crest and at the mid-depth ³He maximum on the eastern flank of the East Pacific Rise, south and west of the present area². Relative to this background water, which is presumably the water entering the hydrothermal system, the ³He/⁴He ratio in sample 7–0 is enriched by 53% (1.99/1.30). The other two plume samples are also enriched in ³He relative to ambient background, but by much smaller amounts. The $\delta(^3\text{He})$ value of 99% measured on sample 7–0 is by far the largest value yet observed in ocean waters, and establishes for the first time a clear connection between the mantle helium in oceanic basalts and the excess ³He observed in the deep oceans, by seawater penetration and circulation in the basalts.

Radon concentrations (Table 1) are reported as 'excess radon' relative to the activity of ²²⁶Ra. The ²²⁶Ra activity was assumed to be 33 d.p.m. per 100 kg from a previous study by this laboratory¹¹ on bottom waters 29 km south-west of the present location in approximately the same depth; rough measurements made on the present small samples were in

Table 1 ^3He and ^{222}Rn measurements on hydrothermal plume and background water on the Galapagos Rift ($\sim 0^\circ 48'\text{N}$, $85^\circ 55'\text{W}$)

Tow no., bottle no.	$\delta(^3\text{He})$ (‰)	Excess ^{222}Rn (d.p.m. per 100 kg)
<i>a</i> Samples with potential-temperature spikes		
7-0*	99.3	191
8-3	35.7	80
8-6	42.6	229
<i>b</i> 'Background water'		
7-1	27.8	39
7-2	—	49
8-1	28.6	—
8-2	30.2	33
8-5	33.4	—
8-7	32.2	—
Mean	30.4 ± 2	40 ± 8

Radon precision = ± 3 d.p.m. per 100 kg (counting error); ^{226}Ra activity assumed = 33 d.p.m. per 100 kg. $^3\text{He}/^4\text{He}$ ratios are accurate to $\pm 0.7\%$ in $\delta(^3\text{He})$.

*The temperature spike for sample 7-0 is shown in Fig. 1 of ref. 10; samples 8-3 and 8-6 were collected from plumes showing smaller temperature spikes.

agreement within the larger errors. The excess radon concentrations in the three plume samples (80–229 d.p.m. per 100 kg) are clearly much greater than the background value of about forty, although they do not scale with the ^3He anomalies. Because of the short radioactive mean life (5.5 d) the ^{222}Rn enrichments in plume waters are probably due to extraction of radon from the basalts, although some contribution from sediment material trapped within the axial fissure cannot be excluded.

Fig. 1 shows the absolute ^3He and ^4He concentrations in the deep tow samples, together with atmospheric solubility values and vectors for addition of atmospheric helium to air-saturated deep water, and for addition of radiogenic ^4He and pure 'mantle helium' (basalt values^{3,4}) to background water on the Galapagos spreading centre. (The 'absolute' concentrations, based on preliminary 'peak-height' determinations for ^4He , are accurate to about 5%; more accurate isotope dilution measurements are in progress.) ^3He and ^4He enrichments in the ambient background water relative to equilibrium solubility represent the net effect of air injection¹² and addition of mantle

and radiogenic helium to deep water during its circulation. The absolute helium anomalies in sample 7-0 are very much larger: ^3He and ^4He concentrations are respectively 75% and 14% greater in this sample than in the mean Galapagos background water.

The added increment of ^3He relative to background water is $5.9 \times 10^{-14} \text{ cm}^3 \text{ STP per g}$ in sample 7-0, an amount approximately equal to the initial solubility equilibrium (Fig. 1) and thus about 0.03% of the ^3He concentration observed in the Red Sea brines. The estimated temperature of inflowing Red Sea brines is $\sim 100^\circ\text{C}$ (ref. 13); thus if the hydrothermal systems in Red Sea and Galapagos basalts are at all similar, a temperature spike of $\sim 0.03^\circ\text{C}$ is predicted for sample 7-0, in reasonable agreement with the signal observed during sampling¹⁰.

In Fig. 1 the vector from the mean background water to sample 7-0 corresponds to addition of helium with $^3\text{He}/^4\text{He} = 9.4 \times 10^{-8}$ or $R = 6.7 R_{\text{Atm}}$, significantly lower than the 'mantle helium' ratio ($R \sim 10 R_{\text{Atm}}$) observed in ocean-ridge basalts^{3,4}, which implies that about 30% of the added helium is radiogenic ^4He generated in the oceanic crust. Helium with $R = 6.7 R_{\text{Atm}}$ would be found in Pacific ridge-basalts after about 30 my of ^4He production (ref. 4), an age far too great for basalts in such close proximity to the rift axis. The holocrystalline portions of pillow basalts exchange trapped gases with seawater on a much shorter time scale than this¹⁴; thus the helium isotope ratios in basalt-seawater hydrothermal systems probably decrease continuously with time as the initial mantle helium component is progressively stripped out by the fluid phase and the radiogenic component grows in. It may therefore be possible to characterise such hydrothermal systems in time and space by detailed mapping of helium isotope ratios in plume samples.

The extreme sensitivity of helium and radon for plume detection is indicated by our estimate that sample 7-0 contained of the order of 0.03% of hydrothermal water. A sample with a 1°C temperature spike would, on this basis, contain helium with $R \sim 5.5 R_{\text{Atm}}$ and a ^{222}Rn activity of $\sim 5,000$ d.p.m. per 100 kg. Radon is of course a radiogenic isotope which is present in very high activity in marine sediments; it is therefore not a completely unambiguous tracer for seawater which has penetrated basalts. The ^3He signature, however, provided unequivocal evidence for the existence of a hydrothermal basalt-seawater system in the axial region of the Galapagos Rift.

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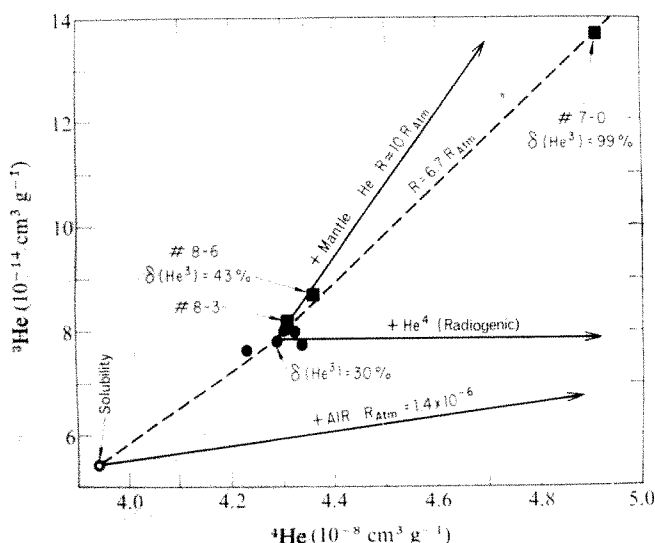
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Fig. 1 ^3He and ^4He concentrations in water samples with temperature spikes (squares) and in ambient 'background water' (circles) on the axial fissure of the Galapagos Rift. The point labelled 'solubility' indicates the equilibrium air saturation concentrations for deep water. ● Normal bottom waters; ■ Hydrothermal plumes.



High explosive analogue of the Tunguska event

THE Tunguska event of 1908, which has been described in detail by Krinov¹, is generally accepted as having been caused by a detonating bolide. Zotkin and Tsikulin² concluded, on the basis of laboratory experimentation, that the event could best be fitted by a travelling wave striking the forest at an angle of 30° to the horizon, culminating in a detonation at a height roughly eight times that of the forest canopy. The pattern of tree blowdown consisted of a radially symmetric zone 35 km in diameter, on which was superimposed a bi-lobed pattern caused by the travelling wave. Stanyukovich and Bronshten³ quote a total energy of 10^{23} erg for the event. In August 1966 an experiment was carried out in a mixed forest of Pine, Spruce and Fir near Hinton, Alberta, Canada, which has some features in common with the Tunguska event. In the experiment a hemispherical stack of TNT, 100,000 lb (50 short tons) in weight, was detonated on the forest floor. The resulting pattern of tree blowdown is shown in Fig. 1.



Fig. 1 Effect of 50-t charge in a mixed forest.

The blowdown zone is 130 m in diameter, and may be compared with the zone of symmetric blowdown at Tunguska. Simple cube root scaling of the dimensions lead to an estimate of 10^3 tons TNT for the Tunguska event. On the basis of a 50% partition of energy, this is equivalent to a 200-kiloton (kt) nuclear event. Taking 1.2 kcal g^{-1} for TNT, the estimated energy for Tunguska is 4×10^{21} erg, two orders of magnitude smaller than the estimate given above. This lower estimate, however, may be a close approximation to the energy of the final detonation, that is to say ignoring the contribution from the travelling wave.

Note, however, that the experiment was a surface explosion, whereas the Tunguska event is believed to be a low 'air burst'. Thus, a refinement of the estimate could be made using the standard height of burst curves. The expected difference in the pattern of blowdown at the full scale—that is, due to a 200-kt detonation at, say, 200 m height—is unlikely, however, to approach an order of magnitude, and will probably be closer to unity. In view of the uncertainties inherent in comparing conventional, nuclear and kinetic energy events in terms of their residual effects, such a refinement of the calculation would be quite unjustified.

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Body-centred cubic cylinder packing and the garnet structure

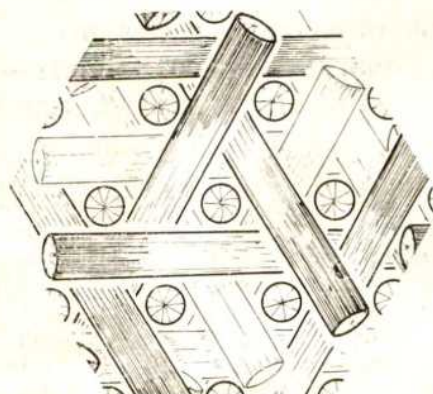
ANALYSIS of the symmetry of periodic packings of identical (circular) cylinders has been found to be useful in analysis of crystal structures. To provide a description of a crystal structure, the cylinders are replaced by rods¹ of atoms or groups of atoms. The densest cubic packing of cylinders is of particular interest: we show how the garnet structure can be described simply in terms of this packing.

Periodic packings of simple geometric objects such as spheres and polyhedra have long had an important role in crystal structure description but little attention has been paid to the problem of cylinder packings in this connection. Periodic packings of cylinders with parallel axes or with axes in parallel planes are fairly easy to visualise, but packings with cubic symmetry are less obvious. One of these, described here, is of special importance to the description of complex cubic structures previously considered enigmatic.

In this packing, cylinders in contact with each other lie with their axes parallel to four cubic $\langle 111 \rangle$ directions. A sketch of a fragment of the packing is shown in the figure. The space symmetry group² of the arrangement is $I4_1/a\bar{3}2/d$ ($Ia3d$) so we propose to call it body-centred cubic cylinder (or rod) packing. The fraction of space filled by cylinders in contact is $\sqrt{3}\pi/8 = 0.680$; this is considerably less than for the densest cylinder packing (which is the familiar honeycomb arrangement with hexagonal symmetry) for which the space-filling fraction is $\pi/\sqrt{12} = 0.907$. Nevertheless it seems to be a safe conjecture that this is the densest cubic packing of identical cylinders.

In applying the cylinder packing to crystal structure description we notice that each cylinder axis lies on a threefold symmetry axis so that a characteristic feature of the symmetry is the presence of four non-intersecting threefold axes. Crystal structures that will lend themselves to description in terms of

Fig. 1 A fragment of body-centred cubic cylinder packing viewed down a trigonal axis.



body-centred cubic rod packing will therefore have this symmetry feature: their space group will be one of the cubic subgroups of $Ia3d$. These are $I43d$, $I4_132$, $Ia3$, $I2_13$, $Pa3$, $P4_132$, $P4_332$, and $P2_13$.

A good example of a crystal structure that can be described as a body-centred cubic rod packing is that of the garnets (symmetry $Ia3d$) typified by grossularite, $\text{Ca}_3\text{Al}_2\text{Si}_3\text{O}_{12}$. The structure³ has been known for fifty years, but has so far resisted a simple description. In this structure the anions are arranged around the threefold axes to form infinite rods of alternating and nearly regular triangular prisms (empty) and octahedra (centred by aluminium ions). Arranging such rods in body-centred cubic rod packing accounts for all the anions in the structure and leaves available sites of eightfold and tetrahedral coordination to accommodate the calcium and silicon ions respectively. The cation positions are fixed by the high symmetry so that an 'ideal' garnet structure can be derived in which the rods consist of alternating regular octahedra and prisms of anions. The coordinates of the 96 anions in the unit cell in the general symmetry-related positions $96h$ are then

$$x = (2\sqrt{3} - 3\sqrt{2} + \sqrt{6} - 2)/8 = -0.0411$$

$$y = (\sqrt{6} - 2)/8 = 0.0562$$

$$z = (3\sqrt{2} - 2\sqrt{3} + \sqrt{6} - 2)/8 = 0.1535$$

very close to those found for real crystals, e.g., for berzeliite⁴ ($x = -0.0391$, $y = 0.0522$, $z = 0.1568$).

Other cubic crystal structures that may be described similarly as a cubic rod packing include those of Th_3P_4 (space group $I43d$), $\beta\text{-Mn}$ ($P4_132$), RbAg_4I_5 ($P4_132$), and bixbyite ($Ia3$). A full description of these and related structures and of rod packing in general is in preparation. It would be of interest also to explore the possibility of cubic rod packings occurring in assemblies of rod-shaped biological molecules or even in the structure of organic matter at a high level such as cellular substructure.

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Behaviour of mercury species in isolated estuarine sediment samples—growth and decay of methyl mercury during storage

WE have investigated the levels of methyl mercury found in mercury-containing sediments from the River Mersey Estuary, England, as part of our work on the determination and transport of mercury in the environment¹. We now wish to draw attention to the unexpected behaviour of sediment-bound methyl mercury on storage. In the initial stages of this study we experienced difficulty in obtaining consistent methyl mercury values; supposedly identical samples analysed at intervals of a few days gave markedly different results. We, therefore, followed the levels of methyl mercury in selected sediments over a period, to

determine if any change was occurring on storage. We found that the amounts of methyl mercury observed in the stored sediments did not remain constant; initially there is a rise in the amount of methyl mercury observed, and then, after about ten days, the amount present begins to decline to levels which in general only approximate to those originally present. We have observed this phenomenon in nearly all of the Mersey sediments samples we have examined; in two cases where the total mercury levels were $< 1.5 \mu\text{g g}^{-1}$, a rise in methyl mercury levels was not detected. In general the sediments were in fact found to contain quite high total mercury levels (usually between 5 and $8 \mu\text{g g}^{-1}$) and this feature may be a requirement for the effect to be observed.

For each experiment, a bulk sample of sediment was taken from the intertidal zone of the River Mersey Estuary. This sample (of several kilograms weight) was immediately homogenised (by vigorous hand mixing) on site, and divided into a number of subsamples, each of about 120 g. Two or three of these were immediately frozen with solid carbon dioxide or liquid nitrogen.

On return to the laboratory, the frozen samples were thawed and analysed for methyl mercury. The values obtained were considered to be the ambient levels present in the sediment in estuarine conditions. The other samples were analysed at regular intervals, and the methyl mercury found was plotted against the time elapsed since they were collected. The samples were stored in the laboratory, in darkness, and samples were also analysed for total mercury as previously described¹. The samples were sealed in glass containers in anaerobic conditions at room temperature (18°C). In this, and in nearly all other cases, we observed that on analysis over a period of about ten days, the initially identical samples in fact revealed quite different methyl mercury contents. During storage over this period the level of methyl mercury increased quite significantly until a maximum level was observed after the ten-day period. This maximum is normally about 2–4 times that present initially, although on occasions increases between 10 and 15 times have occurred. After about 10 days the level of methyl mercury declined to an equilibrium level over a period of about 30–40 days. Samples were taken from three different locations in the estuary: Hale Point (Map Ref. SJ 471808), Fiddlers Ferry (SJ 563865) and Stanlow Marshes (SJ 448788), and showed similar results. The total mercury level was usually between 5 and $8 \mu\text{g g}^{-1}$. Samples were obtained on four separate occasions during 1975 and 1976.

The method used for the analysis of methyl mercury was adapted from that of Uthe, Solomon and Grift². Sediment samples of 2–5 g were extracted with toluene after treatment with copper sulphate and an acidic solution of potassium bromide. Methyl mercury was then back extracted into aqueous sodium thiosulphate. This was then treated with acidic potassium bromide and copper sulphate following which the methyl mercury was extracted into pesticide grade benzene containing approximately $100 \mu\text{g dm}^{-3}$ of ethyl mercuric chloride as an internal standard. The extract was analysed by electron capture gas chromatography using a Pye 104 chromatograph equipped with a nickel 65 detector. The glass column ($1\text{ m} \times 0.4\text{ cm}$) was packed with 5% neopentyl glycol adipate on Chromosorb G (AW-DMCS). Methyl mercury was measured by comparing the peak heights with standards of methyl mercuric chloride made up in the ethyl mercury–benzene solution. The results were calculated as nanograms of methyl mercury per gram of dry sediment. The detection limit was $1\text{--}2\text{ ng g}^{-1}$. Six replicate analyses of a sample gave a mean value of 15.78 ng g^{-1} with range limits of $15.39\text{--}16.34\text{ ng g}^{-1}$. Recovery of 20 ng of methyl mercuric chloride added in aqueous solution to the sediment at the start of the procedure was better than 97%.

It should be pointed out that no mercury species has been added to these samples during or after collection. Therefore the observation of a growth and decay curve similar in both magnitude and time scale to those observed by other workers³⁻⁶, who had incubated sediments with separately added inorganic mercury compounds, was unexpected. There has been no previous report of growth of methyl mercury in stored sediments unless inorganic mercury has been added during the experiment, and these observations are therefore of relevance to analytical work on samples containing methyl and inorganic mercury. It has also been noted that sediments sterilised, normally by autoclaving at approximately 120 °C, did not produce methyl mercury on incubation with inorganic mercury³. This suggested a microbiological origin for the methyl mercury produced from the previous samples.

A control experiment was then carried out in which a large number of identical samples were collected and homogenised. Half of the samples were sterilised by treatment with an approximate 4% w/w solution of formaldehyde. We did not wish to carry out the sterilisation by autoclaving as this would have led to loss of the methyl mercury initially present and also to a substantial reduction in the amount of total mercury present. Several samples of both sterilised and unsterilised sediments were analysed each day. (This duplication was to compensate for any variation between individual samples.) All of the samples were stored at ambient room temperature (18 °C) in the laboratory. It can be seen from Fig. 1 that there is a

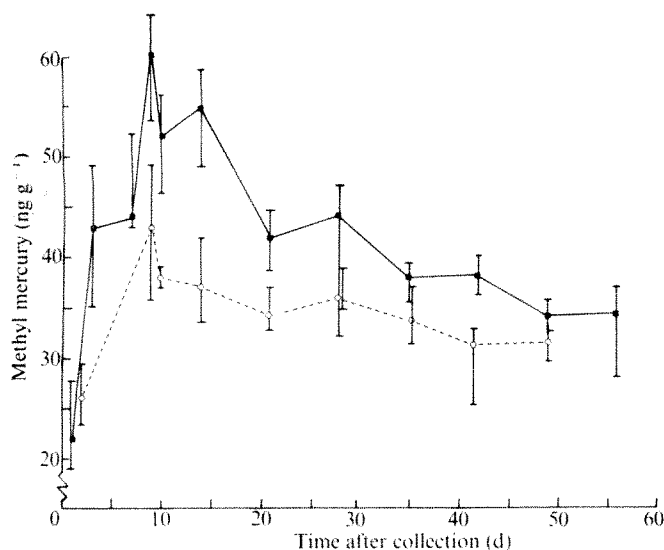


Fig. 1 Analyses of sterilised and unsterilised sediments from Hale Point, for methyl mercury. Total mercury is $7.24 \mu\text{g g}^{-1}$. Results up to day 25 are the mean of eight determinations; results beyond day 25 are the mean of four determinations. Error bars represent range limits for each analysis series. The samples were stored at room temperature (18 °C). —●—, Untreated; ---○---, sterilised samples.

difference in behaviour between the sterilised and unsterilised samples. In addition some of the samples were separately inoculated into various growth media to test for microbiological activity. With the unsterilised samples prolific growth occurred in both aerobic and anaerobic media, whereas the sterilised sediment samples showed no growth in either type of medium. This confirms that the biological activity in the control samples had been destroyed by the sterilisation process used, and Fig. 1 indicates that the initial levels of methyl mercury were not significantly affected.

The growth and decay curve observed in the Fig. 1 for the unsterilised samples was of the same character as we have observed above. But the sterilised samples behaved in an unexpected way in also showing a growth and decay curve, although the maximum level of methyl mercury achieved was significantly smaller.

These observations may be best understood by a consideration of the process of methyl mercury generation in sediments. An equilibrium probably exists between production of methyl mercury from the large excess of inorganic mercury present in the sediments and loss of methyl mercury by evaporation, solution into sediment pore water or by bacteriological degradation⁴⁻⁷. It has also been stated that tidal or other disturbance of natural sediments increases their methylating rates⁸. Such a disturbance would occur on collection and correlates well with our observed initial increase in methyl mercury. When a certain level is reached, however, the methyl mercury then apparently acts as a poison to the methylating species⁹. The mechanism for loss of methyl mercury, however, would continue to operate, possibly in modified form, causing the level to fall gradually. Finally a new equilibrium seems to be reached, but this new steady-state level does not seem to correspond with that found in the estuary in normal conditions.

To explain the smaller growth of methyl mercury in the sterilised samples we suggest that the methylating species (e.g. methyl corrinoids¹⁰) existing in the sediments persists even after the associated organisms that produce it are destroyed by the sterilisation process. This residue then reacts with the reservoir of inorganic mercury present to produce the initial rise again, until it is exhausted and a loss mechanism for methyl mercury predominates. Whereas for the unsterilised samples the methylating species may be biologically generated to allow methylation to continue, this cannot occur for sterilised samples. This scheme fits well the suggested mechanism of methylation as a chemical process with an enzymatic generation of the methylating species¹¹. It should be noted that the loss mechanism for the sterilised samples cannot involve bacteriological activity and this may account for the slightly slower decay rate of methyl mercury in this case. It should also be pointed out that as sterilisation by autoclaving will also destroy the majority of the enzymes and co-enzymes present, no methylation after this method of sterilisation would be expected.

The appearance of excess methyl mercury on incubation of a natural mercury-containing sediment in the laboratory is significant to the previous work on mercury methylation. It suggests that the application of laboratory-derived results directly to natural conditions could, in these cases, be misleading; our analytical results for day 10 if extrapolated directly might lead to the conclusion that natural methyl mercury levels and rates of methylation are much greater than in fact they really are. Work in this area with model or laboratory systems needs to be interpreted with particular caution. As our observations follow the same pattern for samples of different bulk, stored in different containers and at different temperatures, this also suggests that for properly meaningful survey work for methyl mercury the analytical work must be performed within a short time of collection. In the cases where we have not observed the normal pattern, the total mercury concentrations were relatively low. It is possible therefore that these phenomena may only be observable when high concentrations of mercury and organic material are present, that is, in conditions of severe mercury contamination of an already polluted waterway.

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Maya Formative phase radiocarbon dates from Belize

THE Formative phase of Maya civilisation in Central America, until recently thought to date back no further than ~900 BC, has been extended to at least 2,500 BC by a series of radiocarbon dates from the site of Cuello, Belize, on samples from the 1975 excavations¹. The series included two dates of 1,020–1,050 b.c. (1,250–1,300 BC) from upper levels at the site and one date of 2,050 b.c. (2,600 BC) from a midden overlying the buried land surface. (The convention b.c. for radiocarbon years and BC for calendar years using the bristlecone pine calibration curve is followed here; for the calibration schemes used, see refs 2 and 3.) We report here a further series of 16 dates on samples from the 1976 excavations at Cuello, which confirm the early date for the Maya Formative and which also suggest occupation of the site at an even earlier date, ~3,200 b.c. (4,000 BC).

The florescence of Classic Maya civilisation in the first millennium AD, in the arid Yucatan Peninsula of Mexico and the tropical rainforest of Guatemala and Belize to the south, was preceded by a Preclassic or Formative period still of unknown persistence, beginning with the initial settlement of the Maya lands by sedentary farmers. The Formative is defined by the presence of an agricultural economy, based in Mesoamerica in general on maize, beans, avocado, peppers, squash and root crops. The history of plant domestication in Mesoamerica has been taken back to the eighth millennium b.c. by work in Oaxaca⁴ although settled village life is not known before the third millennium b.c. The introduction of pottery, which is a good indicator of settled life because of its fragility and relative non-transportability, is dated between 2,500 and 2,000 b.c. (3,250–2,500 BC) in several regions of Mesoamerica. The emergence of the first recognisably complex society, that of the Olmec, marked by monumental sculpture and public architecture, occurred after 1,200 b.c. (1,500 BC)⁵.

The site of Cuello, located in northern Belize (formerly British Honduras) at approximately 18°05'N, 88°35'W, is a small Classic Maya ceremonial precinct and settlement located on the crest of the low limestone ridge between the parallel courses of the Rio Hondo and the Rio Nuevo, which flow east of north into Chetumal Bay on the Caribbean coast of the Yucatan Peninsula. To the south of the Classic site, so far only cursorily investigated, lies a series of large platforms; one of these, designated Platform 34 on the site map made in 1975–76 by M. Walton, B. Ah and F. Johnson (Fig. 2 in ref. 6), was investigated in 1975 under the supervision of D. Pring, and yielded the first stratified Early Formative cultural sequence in the Maya Lowlands. A second season of excavation in 1976, supervised by S.D. with the assistance of N. Fradgley and G. Kelsey, resulted

in the exposure of a sequence of buildings set around open patios which are, on the basis of the radiocarbon chronology reported here, the earliest known in the Maya area and among the earliest in Mesoamerica.

The samples for dating were collected during excavation from structural, building fill and midden contexts. In general the concordance between stratigraphy and chronology indicates that organic material redeposited in fill was so used within a short time of the original deposition, but in the case of the three earliest dates reported here, which are stratigraphically anomalous, redeposition many centuries later is posited.

The dates obtained on the 1976 excavation samples are shown in Table 1 together with an indication of the context, associated ceramics and major cultural period, and calibration of the dates on the schemes of Ralph *et al.*² and Clark³. They are also shown in Fig. 1 with the range of 1 and 2 s.d. indicated, correlated with the phases of building construction I–VIII elucidated in the excavation, which form a stratigraphic control on the radiocarbon chronology.

Radiocarbon determinations were made in Los Angeles by R.B. and S. de A. and in Cambridge by V.R.S. and A.P.W., using gas proportional detectors filled with purified carbon dioxide produced by the oxidation of the specimens of partially burnt wood from the excavations. Traces of structural plaster were eliminated by treatment

Fig. 1 Radiocarbon chronology for the Formative Period sequence at Cuello, Belize, ranged against a calibrated scale in calendar years (at right, after Clark³) and construction phases O–IX elucidated in excavation. O, old land surface; D, destruction level. The period of erection of the first dated Maya monuments AD 260–300 is also indicated. Laboratory references and uncertainties are shown in Table 1.

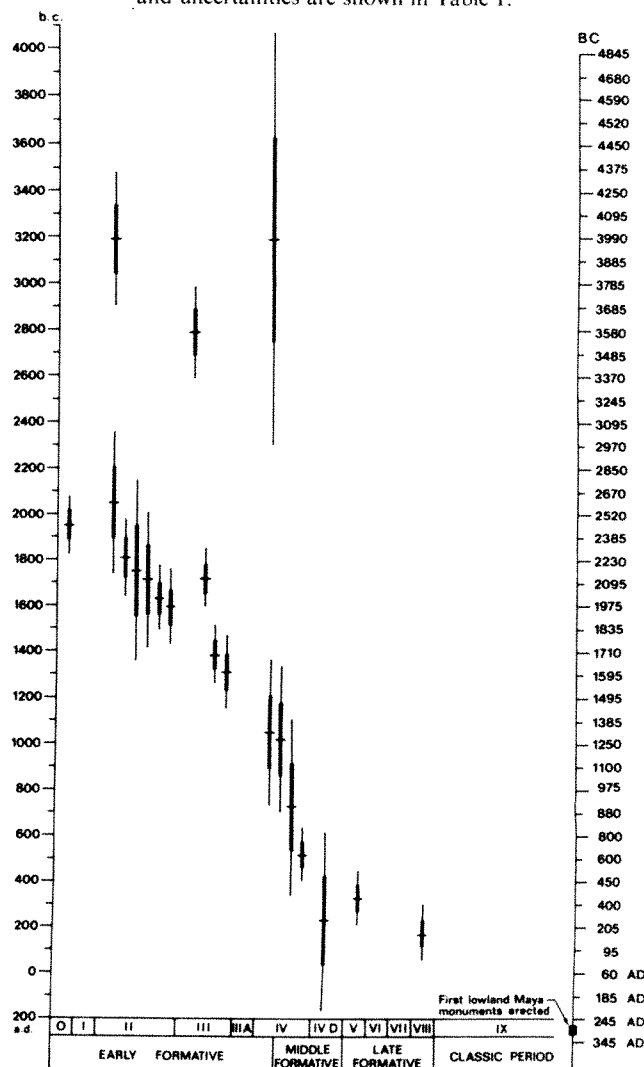


Table 1 Radiocarbon dates from Cuello, Belize, in numerical order of excavation provenance, with context, associated ceramics where present, and major cultural period indicated

Provenance (Op. 17F levels)	Context	Ceramic complex	Major period Maya Valley of area Mexico	Laboratory reference	Conventional ¹⁴ C date b.p. b.c. ±	MASCA 1σ BP	Calibrated date ranges Clark 1σ BP	Clark 1σ BC	Clark 2σ BP	Clark 2σ BC	Archaeological assessment
34	F	Co	Middle/Late Formative Early hori- zon and First Intermedi- ate Period	UCLA-2102f	2280 330 60	2395-2350	2410-2320	460-370	2520-2130	570-180	Acceptable
76	S	Lo		UCLA-2102a	2470 520 60	2740-2450	2750-2430	800-480	2810-2380	860-430	Acceptable
110	F	Lo		UCLA-2102g	5140 3190 445	6420-5450	6430-5470	4480-3520	(6930)-4910	(4980)-2960	Redeposited?*
141	F	Lo		UCLA-2102h	2190 240 195	2435-1940	2430-1870	AD 480-80	2780-1725	AD 830-225	Acceptable
143	S	—		UCLA-2102e	2690 740 70	2960-2120	2950-2790	1000-840	3060-2720	1111-770	Contamination?†
155	S	Sw		UCLA-2102b	4740 2790 100	5630-5340	5640-5335	3700-3385	5775-5250	3825-3300	Redeposited?*
175	F	Sw		Q-1579	3260 1310 80	3660-3460	3685-3445	1735-1495	3830-3320	1880-1370	Acceptable
176	S	—		Q-1574	3670 1720 65	4120-4060	4230-3935	2280-1985	4395-3780	2445-1830	Acceptable
189	D	Sw		Q-1573	3580 1630 70	4090-3990	4415-3840	2465-1890	4735-3580	2785-1630	Acceptable
189	D	Sw		UCLA-2102d	3700 1750 200	4490-3950	4100-3805	2150-1855	4260-3665	2310-1715	Acceptable
197	S	Sw	Early Formative Initial Ceramic Period	Q-1578	3340 1390 65	3820-3590	3785-3520	1835-1570	3940-3400	1990-1450	Acceptable
210	S	Sw		UCLA-2102c	5140 3190 145	6140-5710	6105-5770	4155-3830	6330-5610	4380-3660	Redeposited?*
211	S	Sw		Q-1577	3550 1600 85	4060-3870	4055-3765	2105-1815	4210-3625	2260-1670	Acceptable
219	S	Sw		Q-1576	3660 1710 150	4250-3990	4290-3850	2340-1900	4750-3645	2620-1695	Acceptable
230	O/S	Sw		Q-1571	3900 1950 65	4530-4160	4555-4245	2605-2295	4745-4070	2795-2120	Acceptable
239	M	Sw		Q-1572	3760 1810 85	4390-4100	4360-4035	2410-2085	4515-3960	2565-2010	Acceptable

Contexts F, fill; S, structural; D, destruction; O/S, occupation/old soil; M, midden. Ceramic complexes—Co, Cocos; Lo, Lopez; Sw, Swasey. MASCA refers to Ralph *et al.*

* Datings considered too early for archaeological context.

† Accidental contamination suspected.

with dilute mineral acid, and any humic acid was removed with dilute alkali. Some samples were rather small, and where they yielded insufficient gas to fill the counters, radioactively inert carbon dioxide was added. Samples were counted to a statistical accuracy of 1 s.d. (1σ). The calculated radiocarbon dates quoted are based on the 'Libby' half life for radiocarbon of 5,568 ± 30 yr. Measurement of the stable isotopes ratio ¹³C/¹²C was made on the gas produced from several of the dated samples. The results were very consistent and indicate that samples were of charcoal from normal wood and were not contaminated by carbon of mineral origin.

Our dates consist of nine from the 1975 excavation¹ and 16 from the 1976 excavation. Of these 25, 18 dates are considered to be archaeologically acceptable in their relationship to the site stratigraphy and to each other. Three dates from the 1975 series (UCLA 1985 bc, d and f) are considered too late by more than a millennium to be acceptable, although no certain cause or source of contamination has been found. One 1976 date (UCLA 2102 e) came from a context later suspected of accidental contamination during excavation, and has been set aside as unreliable. Three other dates from the 1976 series (UCLA 2102 b, c and g) are considered too early for the archaeological contexts from which they were recovered, by more than a millennium; these are tentatively ascribed to redeposition of older material, and are discussed below.

The remaining 18 dates provide an internally and stratigraphically consistent radiocarbon chronology for the Cuello site, and through the associated ceramics for the Formative period in the Maya lowlands. Of the 18 dates, 12 are associated with structures and ceramics of the Early Formative Swasey phase, four with the Middle Formative Lopez Mamom phase and two with the Late Formative Cocos Chicanel phase. On the basis of these dates we can propose chronological limits for the three major divisions of the Maya Formative, and also integrate them into the revised stadial model adopted for the sequence in the Valley of Mexico by some scholars⁷.

The central dates for the 12 determinations attributed to the Early Formative range from 2,050 to 1,050 b.c.; the earliest and latest dates in the series have a 1σ of 155 and 160 yr respectively, and taking 2σ as a reasonable outer limit the Early Formative would fall within the period 2,370-730 b.c. The dates for the Middle Formative suggest that the boundary between the two periods does not fall as late as 730 b.c., and we propose that a boundary close to the latest central date be set, at 1,000 b.c. Similarly, at the lower end of the Early Formative we suggest a consonant boundary of 2,000 b.c., although a lowering of this towards 3,000 b.c. with further investigation would not be unexpected in view of the developed ceramic technology at Cuello by 2,000 b.c. and the possibility of earlier occupation mooted below. In terms of calendar years the Early Formative

would span the period 2,500-1,300 bc, coinciding with the limits proposed for the Initial Ceramic period of Mesoamerica in the Valley of Mexico model.

Two other recent radiocarbon dates from Belize also fall within the Early Formative as defined here: a sample obtained by us from the lowest cultural level of the Barton Ramie site in central Belize, associated with ceramics of the early facet of the Jenney Creek ceramic complex, gave a date 3,200 ± 205 b.p., 1,250 b.c., calibrating on 1σ to 1,790-1,320 bc (Q-1575). Another sample, from a wooden post found in the bank of a canal bordering a man-made raised field complex on the Rio Hondo, a short distance west of the Cuello site, yielded a date of 3,060 ± 230 b.p., 1,110 b.c., calibrating on 1σ to 1,650-1,070 bc (1-7877A) (unpublished results of D. Puleston). The Barton Ramie date indicates that by the end of the Early Formative parts of the eastern Maya lowlands were occupied by people making ceramics in a tradition distinct from that of the Swasey complex, while the date from the Rio Hondo raised field suggests that again by the end of the Early Formative at the latest this intensive and organised method of agriculture was being practised in the lowlands. Mollusc evidence from Cuello indicates that swidden agriculture on the limestone ridge was not practised until the beginning of the Middle Formative (unpublished results of L. H. Feldman) so that intensive cultivation may have preceded extensive methods in this region.

The four dates for the Middle Formative are of 1,020, 730, 520 and 235 b.c., the first of these occurring stratigraphically immediately above the final Early Formative levels at the Cuello site and being associated with a burial accompanied by three pottery vessels of transitional Early-Middle Formative type. The 1σ error of 160 yr on this date matches that on the latest Early Formative determination, and the two dates are statistically inseparable. We feel that the phase boundary of 1,000 b.c. is therefore, within the margin of error, acceptable. The date of 235 b.c. does not necessarily indicate an upper limit for the Middle Formative, since the standard deviation of 195 yr is rather large, but a boundary before this date and the earliest Late Formative date of 330 b.c. may be proposed; we suggest that a limit at 400 b.c. will probably lie within the margin of error of a more accurate boundary, when this is established by further determinations. The Middle Formative would then occupy the period 1,300-450 bc in calendar years.

The two dates associated with Late Formative occupation are of 330 and 175 b.c., the latter again not setting a firm upper limit for the period. This upper limit has conventionally been equated with the first appearance of stone monuments bearing hieroglyphic inscriptions in the Maya script, one of the cultural innovations taken as marking the beginning of the Classic period of Maya civilisation. This boundary is currently set between AD 250 and 300 in

the Maya lowlands, and we therefore suggest that the formal bounds of the Late Formative be set at 400 b.c. (450 bc) to AD 250, the latter limit being calculated only in calendar, and not radiocarbon, years from the basis of the evidence by which it has been set.

Although the Middle-Late Formative boundary and the span of the Late Formative might seem to be based on rather less evidence than for the earliest part of the sequence, the limits which we propose are supported by the radiocarbon chronology for the site of Tikal, Guatemala (19 dates in the Middle and Late Formative) and by smaller numbers of dates from other sites including Dzibilchaltun, Aguacatal, Altar de Sacrificios and San Felipe.

We have therefore suggested that, in comparison with the currently accepted framework for the Formative, the Late Formative should begin some 150 yr earlier, the transition between Middle and Early Formative be placed some 500 yr earlier and the formal beginning of the Early Formative be moved back 1,000 yr, in calendar years.

This latter boundary remains the least certain, however. Three of the radiocarbon dates from the 1976 excavations at Cuello are earlier, at 2,790, 3,190 and 3,190 b.c. (3,575, 3,980 bc). The samples of burnt wood from which the dates were obtained occurred in contexts, in two cases structural and in one building fill, from which both later radiocarbon dates and pottery were obtained that fit well into the proposed Early Formative chronology of the site beginning in 2,000 b.c. (2,500 bc). There is no reason for supposing that the early dates are the result of mineral carbon or laboratory error, and we must thus accept that the burnt wood was indeed of the age indicated by the radiocarbon dates. From this it follows, although there is no external corroborative evidence, that the wood must have been reused or redeposited by the Early Formative inhabitants of the site many centuries after it ceased to grow, and in view of the perishability of uncarbonised wood in a tropical lowland soil, probably many centuries after it was charred. If we accept this, there are two methods by which this charring could have occurred: a natural bush fire, and burning by man, on at least two occasions separated by some four centuries. Although there is no internal evidence to support the anthropogenic origin of the burning rather than the natural, such an explanation would correlate with suggested human interference with the vegetation of Peten, Guatemala, 150 km south-west of the Cuello site (unpublished results of F. Wiseman). In addition, the developed nature of the Swasey ceramic complex at Cuello by 2,000 b.c., and the lack of a credible immediate ancestry elsewhere in the Americas (unpublished results of D. Pring) suggest that earlier occupation of the Maya lowlands, if not the Cuello site itself, remains to be discovered. Although the evidence for human presence in the Maya Lowlands in the fourth millennium bc is still sparse and questionable, it is no more so than the totality of evidence for the Early Formative was only two years ago, and the latter is, we would maintain, now tolerably well established.

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Natural selection for energetic efficiency and the relationship between activity level and mortality

A RELATIONSHIP between mortality and metabolic rate may be the basis of a powerful mechanism for the evolution of efficiency in animal locomotion. The ideas presented here were suggested by telemetric observations of brown trout (*Salmo trutta* L.) in their natural environment¹ but may be applicable to a very wide range of animals.

That energetic efficiency is adaptive is an implicit tenet in much recent zoological thought despite the absence of a direct causal link between metabolic efficiency and success in producing offspring. Alexander² describes a simple model for natural selection for efficiency based on the assumption that energy saved in locomotion, for example, is diverted into gonad development so that the efficient genotype produces more offspring. He derives a coefficient of selection of 0.015 for a genotype with non-gonad energy requirements reduced by 1%. This model involves a number of simplifying assumptions and it is clear that increase in gonad products beyond a certain optimum can be disadvantageous³. Selection pressure for efficient energy utilisation may be rather low, any efficiency ratio permitting long term positive energy balance being feasible.

Any animal must function within its 'scope for activity' as defined by Fry⁴. Enforced activity above or below the limits of scope implies certain death. A fish working at moderate power outputs can be assumed to have a small probability of mortality by predation, disease, failure of homeostasis and so on. As the metabolic rate approaches the upper or lower limits of scope so the mortality probability must approach 100%. Metabolic rate (MR) can be normalised to a scale with standard metabolic rate (SMR) as 0 and active metabolic rate (AMR) as 1

$$\frac{MR - SMR}{AMR - SMR} = S$$

Potential mortality per day (M_p) as a function of S probably takes a U-shaped form with M_p equal to 100% at $S < 0$ or $S > 1$ (Fig. 1). Increased time spent at high-activity levels approaching the active metabolic rate may be an important stress and mortality factor in natural populations. Attention has recently been directed to the importance of the cost of reproduction in terms of adult mortality resulting from strenuous activity during the breeding season.⁵

In several marine^{6,7} and freshwater⁸ fish tracking studies it has been shown that high swimming speeds are rare. The total energy requirement for swimming in brown trout has been calculated as 4% of the total ingested energy.^{1,9} This low figure suggests little latitude for fecundity increase by virtue of locomotor energy savings discussed above. Figure 1 shows the typical distribution of metabolic rates of brown trout. Activity in excess of $S = 0.8$ always occupied less than 4% time. The total potential daily mortality (M_{pd}) is equal to the product of potential mortality (M_p) and the time (T_s) at each S level, summed over the whole range of S values:

$$M_{pd} = \sum_{S=0}^{S=1} M_p \times T_s$$

The values of M_p are chosen to give $M_{pd} = 0.1\%$; mortality comparable with that of many natural populations.¹⁰

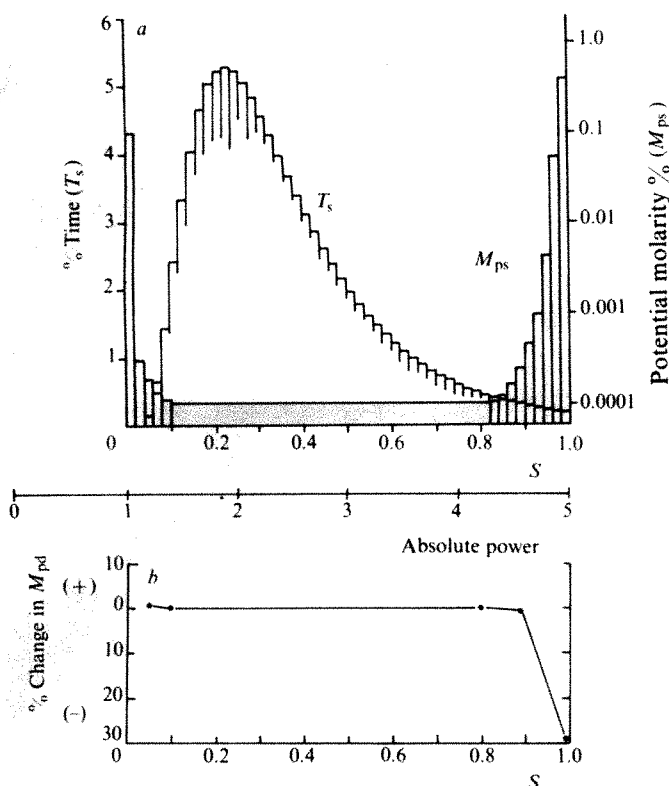


Fig. 1 Activity and mortality in brown trout. *a*, T_s , The typical distribution of time spent at different metabolic rates relative to scope (S , see text). This is based on heart rate telemetry data¹¹ from brown trout in Airthrey Loch, Stirling, Scotland¹. A log-normal distribution was used to fit the curve. $S = 0$ standard metabolic rate. $S = 1$ active metabolic rate. The absolute power or metabolic rate scale is in arbitrary units. M_{ps} , Potential mortality (or failure rate per day) at different values of S . This is a notional distribution based on arguments in the text; no significance is attached to the particular asymmetry or values shown. All calculations are based on the 50 histogram columns shown, each 0.02 S units wide. *b*, Percentage change in total daily potential mortality (M_{pd}) if the total energy expenditure of the fish is reduced by 0.0043% of the total by energy savings at different values of S .

Natural selection consequences of this can be considered. Assume an adaptation appears which reduces drag at a particular speed so that the power required to propel the fish at its maximum cruising speed is reduced by 1%. The time spent at the highest power category can then be replaced by a similar time at a power output 1% lower. In this example the time at $S = 0.99$ is reduced from 0.2% to 0.181% and the time at $S = 0.97$ increases from 0.22 to 0.239. The new value for M_{pd} is 0.0708%; a 29% drop from 0.1% per day. The actual energy saved is only 0.0043% of the total energy expenditure of the fish. If the fish has one year to live until a single spawning the coefficient of selection in favour of the efficient genotype is 0.17 and if it lives to a second spawning a year later the coefficient is 0.244. Thus selection for efficiency can be much more powerful than previously supposed, particularly in long-lived multiple brood animals.

Figure 1*b* shows the change in M_{pd} if the same amount of energy is saved at different levels of S . Reduction of mortality only occurs where the M_{ps} curve has a positive slope. Selection will therefore produce extreme refinement for efficiency at high power outputs but only a small effect at moderate power outputs.

The model in Fig. 1*b* breaks down at low S values since saving energy apparently increases M_{pd} . Energy savings at these S values must consist largely of reduction in standard metabolic rate; that is the $S = 0$ point is shifted to lower absolute power levels. The scope is therefore increased, the crisis of depressed metabolism averted and M_{pd} would decrease.

The penalty of failure to sustain a given activity level is not necessarily death. In the simple case of fish being chased by a

trawl the only escape may be to swim continuously at high speed. Then

$$\frac{\text{Actual mortality}}{\text{Potential mortality}} = C = 1$$

C approaches 1 if: (1) no alternative strategies can be perceived or implemented, alternative resources of food and refuge being important; (2) anaerobic working capacities to escape crises are absent or exhausted; (3) there are no reserves to sustain the animal if it avoids activity and its hazards altogether. The complexities of C should be noted but the main conclusions are not changed. Since C is generally less than 1 the values of M_{ps} in Fig. 1 should be greater than shown. The theory depends critically on the reality of the relationship between M_p and S . Various different shapes of the curve can be envisaged and analogy with engineering reliability theory may be useful in developing the ideas further. Considerations of power capacities and reliability may be more important than energy balance in functional design of animals.

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Chromosomal recombination between *Rhizobium* species

THE basis for the symbiotic specificity whereby legumes are nodulated only by rhizobia of the appropriate cross-inoculation group must reside in genetic determinants in both host and bacterium. To analyse the bacterial determinants, crosses between rhizobia of different cross-inoculation groups are necessary. We show here that the sex-factor R68.45 (ref. 1), which promotes chromosomal recombination in *Rhizobium leguminosarum*² can also mediate gene transfer amongst strains of *R. leguminosarum*, *R. trifolii* and *R. phaseoli*. These species nodulate peas, clover and French beans respectively; thus there is the possibility of identifying bacterial genes involved in host specificity. The only previous report of interspecific conjugation in *Rhizobium* described the transfer of a plasmid that determined host specificity in *R. trifolii* and *R. phaseoli*³. There have also been reports of transformation of genes, including those determining specificity, between *Rhizobium* species^{4,5}.

In *R. leguminosarum* *rif* (rifamycin resistance) and *str* (streptomycin resistance) alleles are cotransferred at high frequencies². We made use of this finding in a series of preliminary crosses with *R. leguminosarum* strain 6591 (*ura-14 met-14 rif str* R68.45) as donor to strains of *R. trifolii* and *R. phaseoli* from the culture collection at Rothamsted Experimental Station, Harpenden, England. Crosses were plated on minimal medium supplemented with streptomycin (200 mg l⁻¹) and rifamycin (25 mg l⁻¹) to select for cotransfer of the *rif* and *str* alleles from *R.*

leguminosarum. One strain each of *R. trifolii* (6601) and *R. phaseoli* (1231) able to receive and express *R. leguminosarum* *rif* and *str* alleles was chosen for further study.

Crosses were made between multiply-marked R^+ *R. leguminosarum* strains as donors and derivatives of 6601 and 1231 as recipients and between *R. trifolii* (R68.45) or *R. phaseoli* (R68.45) with *R. leguminosarum* as recipient. The frequencies of transfer of single alleles and of co-transfer of non-selected alleles in the crosses were measured. Recombinants differing from each parent by two or more alleles could be distinguished from colonies which had arisen by single mutation of either parent to prototrophy or to antibiotic resistance. No unambiguous recombinants (frequency $< 10^{-6}$) were found when R68.45 was absent from both input strains.

The frequencies of transfer of all the selected markers in either direction between *R. phaseoli* and *R. leguminosarum* and for most of the markers in crosses between *R. trifolii* and *R. leguminosarum* were similar to those found in crosses between strains of *R. leguminosarum*² (about 10^{-6} per recipient). As was found in crosses between *R. leguminosarum* strains, more than 80% of recombinants contained the R plasmid, compared with about 1% for non-recombinant recipients.

In Table 1 the frequencies of cotransfer of various pairs of alleles in interspecific crosses are compared with those found in crosses between strains of *R. leguminosarum*. In the interspecific crosses *rif* and *str* were cotransferred at high frequency whether *R. leguminosarum* was donor or recipient. Moreover *rif* and *rif*⁺, *str* and *str*⁺ alleles were transferred to and from *R. leguminosarum* at approximately equal frequencies (results not shown) indicating that donor genes replaced corresponding genes in the recipient rather than that partial diploids were formed.

In many bacterial species *str* and *spc* (spectinomycin resistance) genes are closely linked⁶⁻⁸. This was true for the *spc* allele of *R. phaseoli* (*spc-19*); it cotransferred with *rif* and *str* alleles to *R. leguminosarum* at high frequency. When selection was made for *rif* and *str* transfer from *R. leguminosarum* to *R. phaseoli* carrying *spc-19* about 90% of the recombinants were spectinomycin sensitive (Table 1), indicating the presence of a hitherto undetected *spc*⁺ allele in *R. leguminosarum* closely linked to the *rif/str* region. A *spc* allele of *R. trifolii* was unlinked to *rif/str* (Table 1) but mapped between *phe-1* and *trp-18*, as did the *spc-4* allele of *R. leguminosarum* (unpublished observations).

With the exception of *spc-19* the cotransfer frequencies of pairs of alleles in crosses between *R. phaseoli* and *R. leguminosarum* were similar to those found in crosses between strains of *R. leguminosarum* (Table 1). This was true also for several pairs of alleles in the crosses between *R. trifolii* and *R. leguminosarum*. The frequencies of co-transfer of *ura-14*⁺ and of *met-12*⁺ with *phe-1*⁺ from *R. trifolii* were, however, about tenfold lower than those found in crosses between strains of *R. leguminosarum* which carried these alleles. Also, the absolute frequencies of transfer of *ura-14*⁺ and *met-12*⁺ in the *R. trifolii* × *R. leguminosarum* crosses were about ten times lower than those found for other markers. We do not know whether these alleles are located at another region in *R. trifolii* or whether they do map at the same region as in *R. leguminosarum*, but only that the frequency of recombination in this region is reduced in these interspecific crosses.

If we assume that most of the genes between a pair of alleles were also transferred, integrated into the recipient chromosome and expressed, any essential genes within that region in the recipient must be functionally replaceable by corresponding alleles in the donor. This reinforces the idea that the sequence of genes in the three *Rhizobium* species is very similar. This should greatly facilitate gene mapping in these and perhaps in other species of *Rhizobium*.

To determine whether any of the interspecific recombinants had altered host specificity, individual recombinants inheriting at least two donor alleles were used to inoculate plants; the recombinants between *R. trifolii* and *R. leguminosarum* were tested on peas (*Pisum sativum* cv. Dark Skinned Perfection) and white clover (*Trifolium repens* cv. S100 in the collection at the Welsh Plant Breeding Station, Aberystwyth, Wales) and the recombinants between *R. phaseoli* and *R. leguminosarum* were tested only on peas. Although the frequency of multiple crossover events in recombination is unknown, it is likely that most recombinants received the majority of the genes lying between the two selected alleles. This assumption is supported by the finding that in a cross between *R. trifolii* strain 6647 (*rif spc-20* R68.45) and *R. leguminosarum* strain 1156 (*phe-1 trp-18 str*) all *phe-1*⁺ *trp-18*⁺ recombinants also received the intervening *spc* allele from *R. trifolii*. The recombinants all retained the specificity of the recipient and did not acquire that of the donor (Table 2). Thus it seems that genes determining specificity did not reside in the transferred fragments, or, if they did, that they were not expressed in the heterospecific recipient.

Most studies on interspecific and intergeneric recombination have been done on members of the *Enterobacteriaceae*⁹. The main feature of such crosses is the lowered recombination frequency, although in some cases gene sequences are very similar, as in *Escherichia coli* and *Salmonella typhimurium*. A possible explanation for the general lack of reduction in recombination frequencies in the *Rhizobium* interspecific crosses reported here is that the strains were very closely related and had very similar DNA sequences. Another explanation is that recombination mediated by R68.45 is less dependent on DNA homology than is Hfr-mediated recombination in the *Enterobacteriaceae*. On the basis of numerical taxonomy *R. leguminosarum*, *R. trifolii* and *R. phaseoli* are closely related¹⁰; this was the reason for their choice in this study. Preliminary evidence suggests,

Table 1 Frequencies of cotransfer of pairs of alleles between *R. phaseoli*, *R. trifolii* and *R. leguminosarum*

Alleles cotransferred	a, <i>R. leguminosarum</i> as donor*		
	Mean cotransfer frequency (%)		
	in recipient species		
	<i>R. leguminosarum</i>	<i>R. phaseoli</i>	<i>R. trifolii</i>
<i>rif, str</i>	80	79	85
<i>rif, spc</i> ⁺	NT	89†	<0.7‡
<i>str, rib-2</i>	7	9	13
<i>str, cys-8</i>	7	1	3
Alleles cotransferred	b, <i>R. leguminosarum</i> as recipient		
	Donor species		
	<i>R. leguminosarum</i>	<i>R. phaseoli</i>	<i>R. trifolii</i>
<i>rif, str</i> ⁺	80	94	83
<i>rif, spc</i>	<0.7	83†	<0.7‡
<i>spc, str</i> ⁺	<0.7	88†	<0.7‡
<i>str, rib-2</i> ⁺	7	NT	3
<i>phe-1</i> ⁺ , <i>trp-18</i> ⁺	32	34	32
<i>phe-1</i> ⁺ , <i>met-12</i> ⁺	19	8	<1
<i>phe-1</i> ⁺ , <i>ura-14</i> ⁺	13	14	1
<i>rib-2</i> ⁺ , <i>ade-27</i> ⁺	7	14	11
<i>ura-14</i> ⁺ , <i>trp-18</i> ⁺	1	1	1
<i>spc, trp-18</i> ⁺	NT	<0.7†	75†
<i>spc, phe-1</i> ⁺	NT	<0.7†	41‡
<i>spc, ura-14</i> ⁺	NT	<0.7†	6‡
<i>spc, met-14</i> ⁺	NT	<0.7†	32‡

*Donor/recipient refers to those parental strains in the crosses which respectively carried and did not carry the R-plasmid R68.45.

†*R. phaseoli spc-19* was used.

‡*R. trifolii spc-20* was used.

NT, Not tested

The genetically marked strains of *R. leguminosarum* were derived from strain 300 (ref. 12). The *R. trifolii* and *R. phaseoli* strains were derived from strains 12 and 3644 of the Rothamsted culture collection respectively. Methods for mutant isolation, media and culture conditions have been described¹¹, as have the methods for the analysis of linkage in crosses². In this work 150 colonies were picked from each selective medium and analysed for non-selected markers.

Table 2 Nodulating ability of interspecific recombinants

Donor species	Recipient species	Alleles cotransferred	Number of recombinants tested	Number of plants with nodule	
				Peas	Clover
<i>R. leguminosarum</i>	<i>R. phaseoli</i>	<i>rif</i> , <i>str</i>	15	0	—
<i>R. leguminosarum</i>	<i>R. trifolii</i>	<i>rif</i> , <i>str</i>	37	0	37
<i>R. leguminosarum</i>	<i>R. trifolii</i>	<i>str</i> , <i>trp</i> -14	3	0	3
<i>R. leguminosarum</i>	<i>R. trifolii</i>	<i>str</i> , <i>rib</i> -2	11	0	11
<i>R. leguminosarum</i>	<i>R. phaseoli</i>	<i>str</i> , <i>rib</i> -2	11	0	—
<i>R. trifolii</i>	<i>R. leguminosarum</i>	<i>met</i> -14 ⁺ , <i>spc</i> -20	9	9	0
<i>R. trifolii</i>	<i>R. leguminosarum</i>	<i>phe</i> -1 ⁺ , <i>trp</i> -18 ⁺ , <i>spc</i> -20	15	15	0
<i>R. trifolii</i>	<i>R. leguminosarum</i>	<i>ade</i> -27 ⁺ , <i>rib</i> -2 ⁺	11	11	0
<i>R. trifolii</i>	<i>R. leguminosarum</i>	<i>trp</i> -16 ⁺ , <i>ura</i> -14 ⁺ , <i>spc</i> -20	4	4	0

Peas were inoculated with the interspecific recombinants as described¹⁴. Clover seeds were surface sterilised with sodium hypochlorite, transferred to slopes of water agar in test tubes and nitrogen-free medium¹⁵ containing the inoculum was added. The clover was grown at 26 °C under fluorescent lights. Clover plants were scored for nodulation after 2–3 weeks and peas after 3–4 weeks. Two or three plants were used for each recombinant tested.

however, that *rif* and *str* alleles of *R. leguminosarum* may be transferred by R68.45-mediated recombination to a strain of the apparently more distantly related species¹⁰, *R. meliloti* (unpublished observations). It is relevant that in certain conditions different *Rhizobium* species may occupy the same nodule in substantial numbers¹¹. Such a situation might provide an opportunity for gene exchange in the wild if a sex factor were present in one of the strains.

The ability of fairly large regions of the chromosome to be transferred between strains within a *Rhizobium* species^{2,12} allows contemplation of the possibility of a rhizobium breeding programme whereby recombinants containing favourable attributes from different parental strains (for example competitiveness for nodulation, high levels of nitrogen fixation, tolerance to certain environments) could be constructed. Such strains might prove superior to the parentals for use as inocula. The finding of interspecific recombination means that the gene pool for the formation of such recombinants need not be restricted to the variation encountered within a single species.

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Maturation of prefrontal cortex in the monkey revealed by local reversible cryogenic depression

THE emergence of increasingly complex cognitive functions with age is widely assumed to reflect in part the maturation of connections within the central nervous system, but the neural bases of cognitive development have resisted identification. There is some evidence to indicate that the dorsolateral prefrontal association cortex in monkeys

acquires its adult functional capacities over a protracted span of postnatal development^{1–3}. Most of this evidence, however, has come from studies involving surgical ablation. The study of cerebral development in brain-damaged subjects is complicated by anterograde and retrograde degenerative changes, as well as by the possibility of compensatory neural reorganisation which could occur during the weeks or months between surgical ablation and assessment of behavioural performance⁴. It is now possible to study the functions of specific brain sites without producing permanent brain damage by localised cortical cooling, a method which allows reversible inactivation of restricted regions of cortex in otherwise normal animals. The latter are able to serve as their own controls before and after the episodes of cortical hypothermia^{5–7}. This method has previously been used only in research on mature animals. In this study, local hypothermia was produced for the first time in the dorsolateral prefrontal cortex of young, developing rhesus monkeys during repeated assessment of their performance on a delayed-response task. Such performance in adult monkeys is known to be dependent on the integrity of that region⁸. Our findings provide direct evidence that part of the dorsolateral prefrontal association cortex does not become functionally mature until at least 34–36 month of age, that is, close to sexual maturity in this species.

Seven rhesus monkeys (*Macaca mulatta*) aged 9–36 months were trained to perform a 12-s, two-choice delayed response task. After each monkey reached a criterion performance of 90% correct on four consecutive days, cooling chambers were implanted over a portion of the dorsolateral prefrontal cortex^{9,8} in each hemisphere under pentobarbital anaesthesia. The disk-shaped stainless steel cooling probes (12.5 mm diameter) were implanted epidurally, centered 4 mm above the midpoint of the principal sulcus; the dura remained intact except for two small openings through which insulated bead thermistors were inserted to monitor the subdural temperature. Experimental testing began 1 week after recovery from surgery and consisted of repeated sessions in which the subject's delayed-response performance was assessed before, during and after cooling to 20 °C. Each monkey received 100–200 trials in each of the three conditions (precool, cool, postcool) over the course of 10 sessions.

Intracerebral temperature gradients measured during cooling at terminal stages of the experiment revealed that the areas cooled to 20 °C in all brains was limited to tissue directly beneath the probe and included only the superficial banks of the principal sulcus. The depths of that sulcus remained within 2–3 °C of the normal core temperature. In addition there was no significant hypothermia beyond the boundaries and subjacent white matter of the dorsolateral prefrontal cortex. It should be emphasised that no

significant variation was found in the size or locus of the area cooled in relation to age (Fig. 1). In contrast, the behavioural results were clearly age dependent, as shown in Fig. 2, where the monkeys have been grouped according to age. Dorsolateral prefrontal cooling did not impair delayed-response performance in monkeys 9–16 months of age (Group I). This group made a small number of errors during cooling, but this figure did not differ significantly from the number of errors made during postcool trials. A small but significant increment (7%) in errors due to cooling was first observed in monkeys 19–31 months of age (Group II), but cooling-induced deficits comparable in magnitude (23%) with those seen in adult monkeys^{5,6} did not appear until 34–36 months of age (Group III). The percentage of cooling-induced errors exhibited by Group III monkeys was on the average 2.5-fold higher than that exhibited by the monkeys in Group II.

The pattern of increasing sensitivity to dorsolateral prefrontal cooling, apparent in the comparisons among groups of monkeys at different ages, is also demonstrable in the ontogenetic history of individual monkeys. Thus, one monkey (Fig. 3) manifested a significant but relatively small increase in errors during prefrontal cooling at the age of 31 months, whereas 3 months later this same animal exhibited a 2.5-fold increment in the percentage of errors during cooling, while its control performance during this same interval showed a slight improvement.

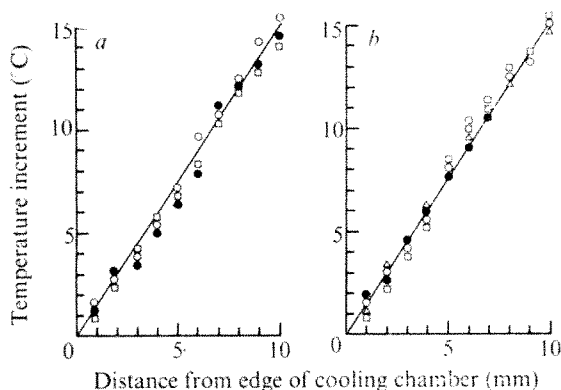


Fig. 1 Intracerebral temperature gradients in two planes, determined with cortical surface beneath the cooling chamber maintained at 20 °C. Shown are the data from four animals aged 9 (□), 18 (△), 34 (●) and 36 (○) months, respectively. *a*, Gradient recorded tangential to the cortical surface. Least square line through data has slope of 1.57 °C mm⁻¹ and correlation coefficient of 0.989. *b*, Gradient recorded perpendicular to cortical surface. Least square line has slope of 1.51 °C mm⁻¹ and correlation coefficient of 0.995. Since the brains of the youngest and oldest monkeys differed in size by no more than 5%, and the probe placement was identical in all, there was no significant difference with age in the amount or location of the tissue cooled.

The finding that prefrontal cooling did not induce deficits in the Group I monkeys is in agreement with previous reports that even complete resections of dorsolateral prefrontal cortex in infancy do not produce impairments on delayed response¹⁻³. The 23% performance decrement observed in the oldest (Group III) monkeys during cooling is, however, substantially less than the nearly 50% decrement usually seen in adult monkeys with prefrontal lesions. This discrepancy is most likely due to the fact that the cortex in the depths of the principal sulcus, normally included in dorsolateral surgical ablations, was not subjected to cryogenic depression. But, as the magnitude of the cooling-induced impairment in the Group III monkeys is nearly identical to that reported in independent studies of adult monkeys carried out with comparable methods^{5,6}, it seems that the Group III monkeys represent the age at which the dorsolateral cortex, exclusive of the depth of the principal sulcus, reaches functional maturity.

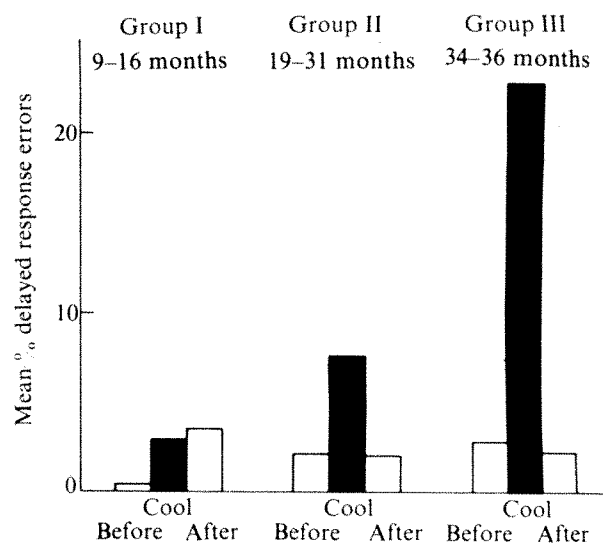
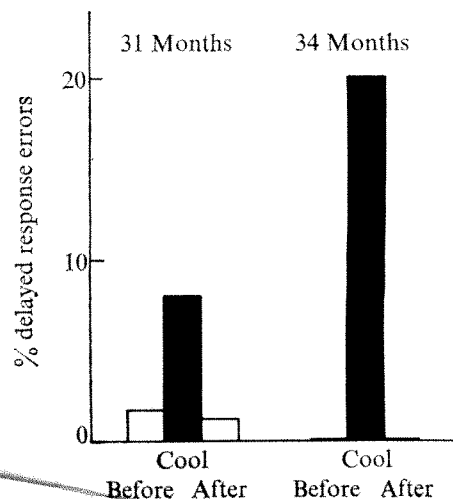


Fig. 2 Delayed response (DR) performance in relation to cryogenic depression of the dorsolateral prefrontal cortex (DLC). Mean % errors on DR trials presented before, during and after DLC cooling for groups of monkeys in the age spans indicated. % DR errors for each animal and condition were subjected to an arcsin transformation, and the transformed data underwent a 3 (age groups) × 3 (DLC cooling condition) factorial analysis of variance with repeated measures on the last-named variable. The main effect for DLC cooling condition was significant ($F(2,10) = 28.1$, $P < 0.001$), as was the age × DLC cooling condition interaction ($F(4,10) = 8.8$, $P < 0.005$). Comparisons of mean DR errors in the different DLC conditions within groups were made using Tukey's (*a*) test with the critical level of $P < 0.01$. Both Groups II (ages 19–31 months, $n = 3$) and III (ages 34–36 months, $n = 2$) made significantly more errors during cooling than either before or after while their performance under the two latter conditions did not differ significantly. In contrast, for Group I (ages 9–16 months, $n = 3$), there were no significant differences in performance among any of the three conditions.

Thus, the present results indicate that a considerable portion of the dorsolateral prefrontal cortex does not participate significantly in the mediation of delayed-response performance during the first 16 months of postnatal life; subsequently, this region participates only minimally until, by 36 months of age, the process of maturation seems to be essentially complete. Previous evidence from ablation studies for the protracted maturation of the dorsolateral prefrontal cortex was open to the alternative explanation that early lesions of this area had failed to result

Fig. 3 % Errors on DR trials presented before, during and after DLC cooling in a single monkey tested at two different stages of development.



in deficits months after surgery because there had been time for recovery of function through compensatory neural reorganisation⁴. This explanation now seems improbable because prefrontal cooling did not produce delayed-response deficits in young, non-lesioned monkeys. In these animals neural reorganisation should not be a factor unless it were assumed that such a process could be stimulated by cortical hypothermia within the course of a testing session.

The lack of dependence on the dorsolateral prefrontal cortex for delayed response behaviour in young monkeys, shown now both in studies of surgical and functional ablation, contrasts with the dependence for such behaviour, from infancy through sexual maturity, on certain brain regions other than the dorsolateral prefrontal cortex, such as the orbital prefrontal cortex^{1,9}, the anterodorsal caudate nucleus¹⁰ and the mediodorsal nucleus^{4,11}. Our results suggest that the dorsolateral prefrontal cortex assumes its mature functions by augmenting rather than replacing these other antecedent neural mechanisms, since there is no evidence of disturbance in the basic ability to perform delayed response during the transition from minimal to maximal dependence upon the dorsolateral prefrontal cortex.

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Gene dosage for isocitrate dehydrogenase in mouse embryos trisomic for chromosome 1

It is generally assumed that changes in chromosome number are reflected in gene dosage effects for loci located in the extra or missing chromosomes. Although such effects have been well substantiated in *Drosophila*¹, data for mammalian aneuploidy are scarce. Quantitative gene dosage effects have been demonstrated for several X-linked enzymes in oocytes of mice with X-chromosome aneuploidy^{2,3} and for malic enzyme in mice with partial trisomy of chromosome 7 (ref. 4). In man, similar effects for superoxide dismutase-1 (W. W. Feaster, L. W. Kwok and C.J.E., unpublished and ref. 5), adenine phosphoribosyltransferase⁶, purine nucleoside phosphorylase⁷ and erythrocyte acid phosphatase⁸ have been observed in cells aneuploid for chromosomes 21, 16, 14 and 2, respectively. Aneuploidy for chromosome 21 also affects responsiveness to interferon⁹, and XYY cells have been claimed to have increased amounts of H-Y surface antigen¹⁰. Since the detection of more than 30 metacentric (Robertsonian translocation) chromosomes in wild and laboratory strains^{11,12} and the development of a mating scheme which utilises such translocations for the production of aneuploid embryos in relatively high frequencies^{13,14}, the mouse has become particularly useful for gene dosage studies. We have used

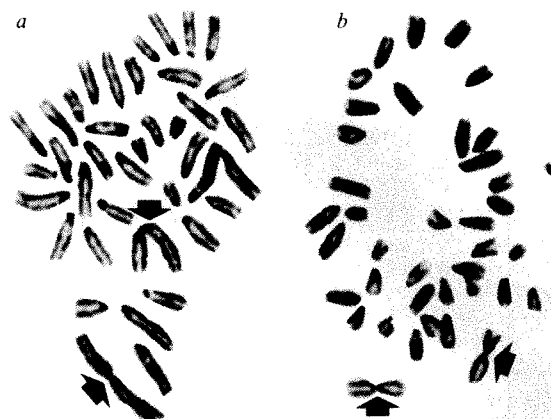


Fig. 1 Metaphase spreads from membranes of trisomic mouse embryos prepared as described by Evans *et al.*¹⁵. a, Trisomy 1; b, trisomy 12. The two metacentric chromosomes indicative of the trisomic state are indicated by arrows in each spread.

this system to demonstrate the dosage effect for supernatant isocitrate dehydrogenase (Id-1) which results from trisomy for chromosome 1.

Males doubly heterozygous for two different metacentric chromosomes which contain chromosome 1, Rb1(1.3)Bnr and Rb10(1.10)Bnr, were obtained by mating animals homozygous for Rb1 with Rb10 homozygotes. To produce embryos trisomic for chromosome 1, heterozygous males were mated with mature white Swiss (WS) females (Simonsen Laboratories), and the time of mating was confirmed by observation of vaginal plugs. For control experiments, embryos trisomic for chromosome 12 or chromosome 19 were generated by using Rb5(8.12)Bnr/Rb9(4.12)Bnr and Rb1(5.19)Wh/Rb163(9.19)H heterozygotes, respectively. These aneuploid embryos are known to be viable until day 14 or 15 of gestation¹⁵. The pregnant females were killed on day 12 or 13, and the embryos (with membranes) were removed. The chromosome constitution of each embryo was determined by examination of metaphase spreads made after short term incubation of the membranes in colcemid as described before¹⁵. Trisomic embryos were identified by their two metacentric chromosomes and total of 41 chromosome arms. Because monosomic embryos die at or shortly after implantation¹⁶, all other karyotypes with only one metacentric chromosome are euploid. As Table 1 shows, between 15 and 42% of the embryos were trisomic. Representative metaphases from trisomic embryos are shown in Fig. 1.

While the membranes were being prepared for chromosome analysis, the individual whole embryos were homogenised and analysed for enzyme activity spectrophotometrically and, when appropriate, by starch gel electrophoresis. The results of assays for Id-1 activity in embryos trisomic for chromosome 1, 12 or 19, and for glucose-6-phosphate dehydrogenase (G6PD, X-linked) and 6-phosphogluconate dehydrogenase (*Pgd*, chromosome 4) in trisomy 1 embryos are summarised in Table 1. Control embryos were the karyotypically normal embryos from the same litters as the trisomics. Trisomy 1 was associated with 1.53 ± 0.04 (s.d.) times the control activity of Id-1, but neither G6PD or *Pgd* activity was significantly different from the control. Thus trisomy 1, with three chromosome 1 instead of two, results in the expected 3:2 increase in activity of chromosome 1-determined Id-1, but not of enzymes coded by other chromosomes; trisomy for either of two other chromosomes does not affect the activity of Id-1.

Given the observed Id-1 activity in trisomy 1, it would

Table 1 Enzyme activities in trisomic embryos

Mating	Chromosome	Trisomy Frequency	Enzyme activity in trisomic embryos (ratio to normal)		
			Id-1	G6PD	PGD
WS × Rb1Bnr/Rb10Bnr	1	14/91 (15%)	1.53 ± 0.04 (6)	0.96 ± 0.05 (5)	0.94 ± 0.26 (5)
WS × Rb5Bnr/Rb9Bnr	12	7/23 (30%)	1.06 ± 0.05 (4)		
WS × Rb1Wh/Rb163H	19	11/26 (42%)	0.98 ± 0.06 (7)		

Results are expressed as mean $\pm$ s.d. of the ratios of specific activities based on soluble protein. The number of determinations is shown in parentheses. Each embryo was individually homogenised in a plastic tube (6 × 50 mm) with a glass homogeniser in 0.05 ml 0.25 M sucrose in 0.01 M phosphate buffer, pH 7.0. Another 0.05 ml sucrose-phosphate was added, and the homogenate was then centrifuged at 27,000g for 20 min at 4 °C. The supernatant fluids were used for enzyme assay and protein determinations. Id-1 was assayed as described by Lowry and Passonneau¹⁸, and G6PD and PGD as described by Glock and McLean¹⁹.

be expected that each of the three genes for Id-1 contributes equally to the total product. To test this directly, the enzyme produced by trisomic and normal embryos was subjected to starch gel electrophoresis. Since Rb1Bnr carries the *b* (fast) allele at *Id-1* and Rb10 the *a* (slow) allele, the Rb1/Rb10 heterozygote is electrophoretically *ab*. Depending on the allele contributed by the Swiss female, which can be either *a* or *b*, the genotype for the Id-1 of the trisomic embryos will be either *aab* or *abb*. Because Id-1 is a dimer, the possible enzyme products are *aa*, *ab* and *bb*. If each gene is functionally equal, the expected ratios of the three are 4:4:1 (if the embryo is *aab*) or 1:4:4 (if *abb*). Normal embryos will be *aa*, *bb* or *ab*, and the electrophoretic patterns will show either pure *aa*, pure *bb*, or *aa*, *ab* and *bb* in a 1:2:1 ratio. As Fig. 2 shows, patterns compatible with the expected trisomic and normal patterns were observed. Furthermore, no patterns suggestive of the presence of three operative *Id-1* genes were observed with embryos trisomic for chromosome 12. Thus the biochemical data are consistent with an exact and specific dosage effect for Id-1 in trisomy 1.

With the large number of mouse translocations which have been identified and which are being placed on inbred backgrounds^{13,17}, it will soon be possible to produce and investigate embryos and cell lines derived from them which are trisomic for most of the mouse autosomes. This approach, of which this report is an example, should permit a systematic and refined examination of the molecular and developmental consequences of chromosome imbalance.

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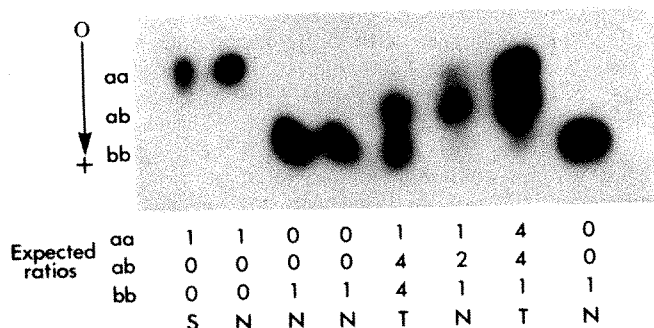


Fig. 2 Electrophoretic patterns of Id-1 from normal (N) embryos and embryos trisomic (T) for chromosome 1. Electrophoresis was carried out in an 11% vertical starch gel for 20 h at 4 °C at a constant voltage of 120 V using the citrate-phosphate buffer system (No. 3) of Ruddle and Nichols²⁰. The gels were stained as described by Henderson²¹. The origin (O) is at the top and the samples were run to the anode (+). The two trisomic embryos show patterns compatible with the expected 1:4:4 and 4:4:1 ratios for the *abb* and *aab* genotypes, respectively. The five normal embryos display the pure *a*, pure *b*, or 1:2:1 patterns compatible with *aa*, *bb* or *ab* genotypes, respectively. The standard (S) for the *a* (slow) allele was obtained from a liver homogenate from a BALB/c mouse.

Heparin-like inhibitor of blood coagulation in normal newborn

THE level of the blood coagulation factors in the newborn is known to be markedly lower than that in the adult. Generally this is attributed to a lack of synthetic capacity of the liver and a deficiency in vitamin K. We describe here a re-investigation of this problem making use of recently developed methods that determine both the coagulation factors and the abnormal proteins induced by vitamin K absence (PIVKAs). We found low coagulation factor activity in the newborn but no evidence for vitamin K deficiency. In more than half the samples a hitherto unrecognised inhibitor of blood coagulation could be demonstrated. At 3 d of age this inhibitor was found in all babies investigated. On the basis of its properties in blood coagulation tests the inhibitor could not be distinguished from heparin.

In 43 healthy newborns a 20-cm piece of umbilical cord was clamped directly after birth and 9 ml of blood was withdrawn by puncture from the vena umbilicalis into a polypropylene syringe containing 1 ml of a solution containing HEPES 0.3 M, trisodium citrate 0.1 M, sodium azide 15 mM, Trasylol (Bayer) 50 U, pH 7.3. This mixture effec-

tively prevents fibrinolysis. Plasma was obtained by centrifugation and spun platelet poor by a second centrifugation (30 min 4 °C, 20,000g). It was frozen in 1-ml aliquots and stored at -20 °C until further use. In these plasmas we determined clotting factors V, VII, and X and prothrombin by a one-stage procedure and prothrombin also by a two-stage procedure (Table 1). Experimental methods were as described in ref. 1.

In 27 of the 43 plasmas the disappearance rate of thrombin in the two-stage prothrombin assay was significantly (>5 times) greater than in normal human adult plasma. In the 16 others it was no more than 1.8 times greater than normal. On the basis of this observation we divided the plasma in an inactivated and a non-inactivated group. We found no significant differences in antithrombin III, α_1 -antitrypsin, α_2 -macroglobulin, antiplasmin, C₁-esterase inhibitor and inter- α -trypsin inhibitor activities between inactivated and non-inactivated plasma samples: we therefore conclude that none of these proteins are responsible for the inactivation. No fibrinogen degradation products could be detected in either of the plasmas. Pooled inactivated and pooled non-inactivated plasma was diluted 20-fold with distilled water; isoelectric precipitation at pH 5.2 yielded a fraction containing prothrombin but no thrombin inhibitors. In such fractions made from both plasma types, thrombin generated normally and was not inactivated. In the presence of normal plasma the thrombin thus generated inactivated normally. This excluded the possibility that the rapid inactivation was due to an abnormality of the (pro)thrombin molecule in the inactivated plasma.

Both procoagulants and thrombin inactivating proteins in inactivated plasma were precipitated by phosphotungstic acid (1/3 volume of 10% phosphotungstic acid in 0.67 N H₂SO₄). After neutralisation and removal of the phosphotungstic acid, the resulting fraction accelerated thrombin inactivation in normal plasma by a factor five to seven, and lost its inactivation enhancing properties on addition of 12 μ g ml⁻¹ protamine sulphate. No such fraction could be obtained from the non-inactivated plasma. Inactivation of thrombin in inactivated plasma could be prevented by addition of 14 μ g ml⁻¹ of protamine sulphate whereas such addition had little or no effect on non-inactivated plasma.

Heparin is known to potentiate thrombin inactivation by antithrombin III, to be neutralised by protamine sulphate and not to be precipitated by phosphotungstic acid².

We conclude that the inactivated plasma contains heparin or a substance with heparin-like properties. The source of this substance is presumably in the newborn. It could not be demonstrated in maternal blood or in the perfused placenta. Five children who did not show enhanced inactivation in

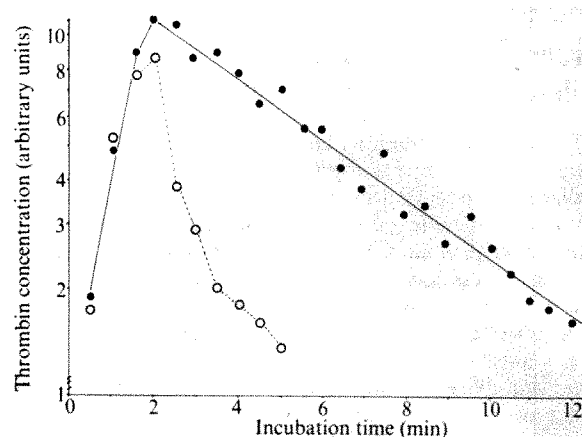


Fig. 1 Thrombin generation and breakdown in pooled newborn plasmas with (○) and without (●) inactivating substance. Reaction mixture: 0.2 ml plasma, 0.1 ml human brain thromboplastin, 1.7 ml Michaelis buffer pH 7.2, 1 ml CaCl₂ 33 mM. At the times indicated 0.1 ml of the reaction mixture was subsampled into 0.1 ml of BaSO₄ adsorbed plasma diluted 1:5 with Michaelis buffer (pH 7.2). From the clotting time obtained the thrombin content was calculated¹.

the cord blood did show this activity in blood taken from the vena cubiti on day 3 of life. The 11 others from the non-inactivated group were not tested.

Presumably this heparin-like substance is produced in organs that are hardly perfused before birth, but will have an active circulation afterwards (that is, the lungs). It may serve to prevent thrombosis in these organs. In this context it is interesting to note that complete deficiency of antithrombin has never been observed although several series of (possibly heterozygous) patients with about a half normal amount of antithrombin III have been reported³. Complete absence of antithrombin III which would prevent the action of the heparin-like substance might be a lethal condition.

We found no differences between the inactivated and non-inactivated groups in any of the coagulation factors tested. More interesting, there was no discrepancy between the prothrombin contents found with the one-stage and the two-stage assay (Table 1). This indicates that no proteins induced by vitamin K absence (PIVKAs) are present in the plasma of the newborn⁴. This is corroborated by the fact that neither in the staphylocoagulase test nor in the Echis Carinatus test nor in a one-dimensional immuno electrophoresis an excess of prothrombin activity was observed whereas the PIVKA when present would co-estimate in these tests (Table 1, ref. 1).

We concluded that, contrary to current beliefs, the newborn has no vitamin K deficiency but is 'anticoagulated' by heparin or a heparin-like substance.

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Table 1 Determination of clotting factors in cord blood

Factor	Method	Blood activity (%)	
		I	Non-I
Antithrombin III	CIE	62 ± 8	61 ± 12
α_1 -Antitrypsin	CIE	74 ± 12	81 ± 15
α_2 -Macroglobulin	CIE	104 ± 20	110 ± 16
Antiplasmin	CIE	49 ± 9	49 ± 8
C ₁ esterase inhibitor	CIE	53 ± 11	58 ± 12
Inter- α -trypsin inhibitor	CIE	64 ± 14	65 ± 13
II	one-stage	51 ± 12	47 ± 10
II	two-stage	48 ± 12	47 ± 11
II	Echis Carinatus venom	54 ± 11	49 ± 9
II	staphylocoagulase	48 ± 8	48 ± 10
II	CIE	53 ± 15	50 ± 16
V	one-stage		
VII	one-stage	43 ± 12	44 ± 10
X	one-stage	31 ± 15	30 ± 7

Values are means ± s.d. of the blood activities as % of pooled blood from 30 normal adults. I, group showing rapid thrombin inactivation ($n=27$); Non-I, group showing normal thrombin inactivation ($n=16$). CIE, crossed immunoelectrophoresis.

Abrogation of reaginic antibodies with modified allergens

ALLERGIC diseases such as hay fever, extrinsic asthma, drug hypersensitivities and some forms of urticaria are mediated by allergen-specific antibodies of the IgE class, known also as reagins. Specific suppression of the IgE antibodies to haptens and protein antigens has been achieved recently by injection of conjugates of haptens with non-immunogenic carriers¹⁻⁵ or by appropriately modified allergens⁶⁻⁸. Here we describe a new method for converting a well defined protein antigen, such as ovalbumin (OA), or the mixture of the non-dialysable allergenic constituents of the aqueous extract of ragweed pollen (RAG), to tolerogenic and non-allergenic products which are capable of abrogating the primary as well as ongoing IgE responses to the corresponding native allergens in B₆D₂F₁ mice and rats. Our method involves the covalent coupling of the allergens to the non-immunogenic, hydrophilic polymer, polyethylene glycol (PEG); this approach was inspired by the report that coupling of PEG to catalase and bovine serum albumin resulted in loss of the immunogenicity of these proteins⁹.

In the present study two batches of PEG (Fisher Scientific Company, New Jersey, USA) with average molecular weights of 6,000 and 20,000, referred to as PEG₆ and PEG₂₀, were coupled to OA and RAG using cyanuric chloride as the coupling reagent¹⁰. After a number of trials, it was shown that PEG-antigen conjugates, possessing the required tolerogenic capacity, were prepared by adding cyanuric chloride (0.2 g in 3 ml of *N,N'*-dimethylformamide) to a mixture of 0.4 g of PEG dissolved in 6 ml of 0.5 N NaOH and 0.1 g of OA or RAG in 5 ml of phosphate-buffered saline, pH 7.2. These conjugates were separated from the original reactants by filtration through Sepharose 4B. The chemical characteristics of these conjugates were not studied in detail in these exploratory investigations and chemically well defined PEG-antigen conjugates, differing in the number and molecular weight of PEG molecules grafted on to each antigen molecule, are currently being synthesised.

Optimal anti-DNP and anti-OA IgE responses were induced in B₆D₂F₁ mice and in Chester Beatty rats and IgE responses specific for RAG, antigen E of RAG and the antigens of the extract of *Ascaris suum* (Asc) were induced in B₆D₂F₁ mice by procedures similar to those described previously^{4,11}. Serum IgE levels were determined by passive

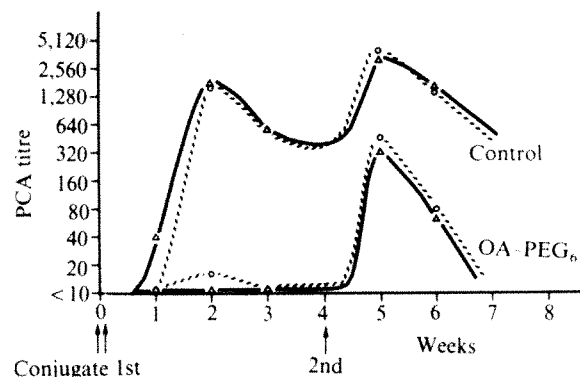


Fig. 1 Suppression of the reaginic antibody response with OA-PEG₆. Test mice were injected i.v. with 1 mg of OA-PEG₆ 4 h before receiving the first sensitising dose of DNP-OA on day 0. The mice received i.p. a second sensitising dose of antigen on day 28 without further administration of the conjugate. Δ — Δ , Anti-OA, IgE response; $\circ$ — $\circ$, Anti-DNP IgE response.

cutaneous anaphylaxis (PCA) assays in random-bred hooded rats⁴.

Intravenous (i.v.) administration of 1 mg of OA-PEG₆ into mice 4 h before immunisation with 1 μ g of DNP₃-OA in Al(OH)₃, referred to as the sensitising dose of DNP-OA, suppressed completely the primary anti-DNP and anti-OA IgE responses and these mice responded very poorly to a further sensitising dose of DNP-OA (Fig. 1). By contrast, injection of 1 mg of free PEG₆ or PEG₂₀, or of OA which had been treated with cyanuric chloride in absence of PEG, had no effect on the ability of the animals to mount IgE responses to DNP-OA. The specificity of the immunosuppression was demonstrated by the results listed in Table 1. Thus, whereas treatment of mice with OA-PEG₆ suppressed profoundly their ability to produce IgE antibodies to both DNP and OA on sensitisation with DNP-OA, it did not affect their capacity to produce IgE antibodies to both DNP and Asc on immunisation with DNP-Asc. Hence, the mechanism of immunological suppression induced by OA-PEG seems to operate at the level of cells interfering with the normal cooperation between helper T cells and B₆ cells¹².

In relation to the potential use of PEG-allergen conjugates for the immunotherapy of allergic individuals, it is to be noted that i.v. injection of OA-PEG conjugates into mice presensitised with DNP-OA led to the suppression of their ongoing anti-DNP and anti-OA reaginic responses.

Table 1 Specificity of immunosuppression with OA-PEG

Group	Treatment		Day	Primary immunisation			Day	Secondary immunisation		
	Day 0	Day 28		Anti-DNP	Anti-OA	Anti-Asc		Anti-DNP	Anti-OA	Anti-Asc
Control A*	DNP-OA	DNP-OA	14	1,000	1,000	NT	35 42	3,310 1,280	3,500 3,020	NT NT
Test A†	OA-PEG ₆ plus DNP-OA	DNP-OA	14	< 10	< 10	NT	35 42	80 480	480 860	NT NT
Control B*	DNP-Asc	DNP-Asc	14	890	NT	200	35 42	1,000 640	NT NT	1,500 840
Test B‡	OA-PEG ₆ plus DNP-Asc	DNP-Asc	14	600	NT	180	35 42	1,650 780	NT NT	1,700 640

*Mice in control groups A and B received on each of days 0 and 28 a sensitising dose of DNP-OA or 10 μ g of DNP-Asc in Al(OH)₃, respectively.

†Mice in test group A received an i.v. injection of 800 μ g OA-PEG₆ 4 h before administration of the sensitising dose of DNP-OA on day 0 and were challenged with the same dose of DNP-OA on day 28.

‡Mice in test group B received an i.v. injection of 800 μ g OA-PEG₆ 4 h before sensitisation with 10 μ g DNP-Asc in Al(OH)₃ on day 0 and were challenged with the same dose of DNP-Asc on day 28.

§Groups of four to five mice were bled on day 14 for the determination of the primary IgE response and on days 35 and 42 for assessing the level of IgE antibodies in the secondary response; the sera of each group were pooled prior to PCA testing (NT, not tested).

Table 2 Induction of tolerance in rats with OA-PEG₂₀

Group	Treatment*	Rat no.	PCA Titres†		Systemic Reaction‡
			Anti-DNP	Anti-OA	
Control	DNP-OA + Al(OH) ₃ + 10 ¹⁰ <i>B. pertussis</i>	1	90	80	Death in 24 min
		2	260	100	Death in 33 min
		3	260	100	Death in 36 min
		4	270	80	Death in 28 min
Test	OA-PEG ₂₀ and DNP-OA + Al(OH) ₃ + 10 ¹⁰ <i>B. pertussis</i>	5	<10	<10	No symptoms
		6	20	20	No symptoms
		7	<10	<10	No symptoms
		8	<10	<10	No symptoms

*All rats were sensitised i.p. with 1 µg DNP₃-OA in presence of 1 mg Al(OH)₃ and 10¹⁰ *Bordetella pertussis*; the rats in the test group received an additional i.v. injection of 2.5 mg of OA-PEG₂₀ 4 h before sensitisation.

†The PCA titres refer to the reaginic antibodies in sera of rats 14 d after sensitisation.

‡Rats were challenged i.v. with 2 mg of OA in 1 ml PBS 14 d after sensitisation.

Similarly, the administration of either RAG-PEG₆ or RAG-PEG₂₀ into mice, which had been sensitised with RAG, abrogated their ongoing anti-RAG IgE response and further sensitisation with RAG did not restore their anti-RAG IgE antibody levels (Fig. 2). The results given in Table 2 indicate that tolerance was also induced in rats by i.v. injection of OA-PEG₂₀ 4 h before sensitisation with DNP-OA and, most significantly, whereas rats made tolerant by this treatment did not show any discomfort on i.v. challenge with OA, the control-sensitised rats died of anaphylaxis after receiving OA.

In addition to being tolerogenic, PEG-allergen conjugates were shown to be devoid of the ability to react with antibodies to the unmodified allergens. Thus, these conjugates failed to combine with antibodies to the native allergens, as proven by their inability (1) to neutralise *in vitro* IgE antibodies to the unmodified allergens (demonstrated by PCA as described in ref. 13), (2) to give precipitin bands with rabbit antisera specific for the native allergens, (3) to induce anaphylaxis in sensitised rats and (4) to trigger PCA reactions in skin sites sensitised with IgE antibodies to the native allergens. Moreover, it is noted that the lack of allergenicity of RAG-PEG₂₀, was also confirmed with respect to human reagins to RAG. Thus, in experiments involving the direct skin testing of a RAG-sensitive individual and PCA testing of a rhesus monkey sensitised with a reaginic serum of a patient allergic to RAG, RAG-PEG₂₀ was shown to be essentially devoid of the allergenicity of native RAG. Hence, PEG conjugates of common allergens may prove to be useful therapeutic agents for the abrogation of IgE-mediated allergies without unleashing anaphylactic reactions. The lack of allergenicity and antigenicity of these tolerogenic PEG conjugates was also paralleled by their non-immunogenicity, that is, their

inability to induce an antibody response in normal mice in absence of adjuvants.

After induction of tolerance with PEG-antigen conjugates in mice, the state of unresponsiveness could be maintained even after transfer of tolerised spleen cells into X-irradiated recipients, that is, challenge of the recipients with a sensitising dose of the antigen failed to elicit a secondary response. Furthermore, the results of more recent experiments indicate that the suppressive effects due to PEG-modified antigens were caused by suppressor cells specific for the determinants of the carrier portion of the native antigens. This observation is in agreement with the recent demonstration⁷ that urea-denatured antigen E of RAG also generated suppressor T cells and one may, therefore, suggest that an antigen in a non-immunogenic form favours the proliferation of T suppressor cells. The cellular mechanism underlying the observed suppression of the IgE response by PEG-antigen conjugates remains to be elucidated with chemically better defined PEG-antigen conjugates. We expect that the number and molecular weight of PEG molecules grafted on to each antigen molecule would affect the structure of the determinants of the antigen as well as their accessibility to the free and cell membrane-bound immunoglobulins and to receptors on helper, suppressor and accessory cells involved in the overall immune response.

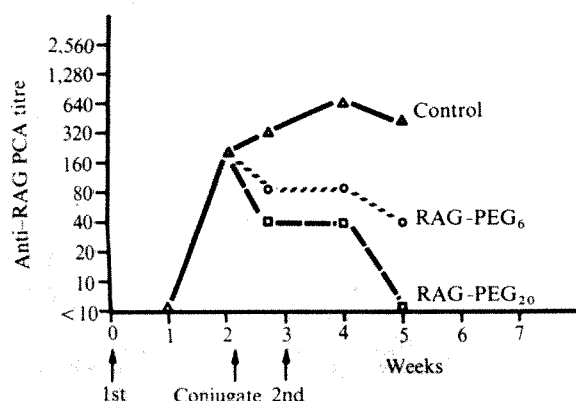
These studies were supported by the Medical Research Council of Canada.

Note added in proof: It has been recently reported¹⁴ that coupling of antigen E of RAG to PEG of molecular weight 2,100 resulted in pronounced reduction of the allergenicity and immunogenicity of this antigen. No indication was given if this conjugate was immunosuppressive, which is the most prominent feature of the conjugates prepared by us.

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Fig. 2 Abrogation of the reaginic antibody response to ragweed allergens (RAG). Test mice were immunised i.p. with 10 µg of RAG in presence of Al(OH)₃ on day 0 and 800 µg of either RAG-PEG₆ or RAG-PEG₂₀ were injected i.v. on day 15. The mice received i.p. a second sensitising dose of the antigen on day 21 without further administration of the conjugate.



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Retinyl acetate inhibits mammary carcinogenesis induced by *N*-methyl-*N*-nitrosourea

THE use of pharmacological agents to prevent development of epithelial cancer is attracting increasing attention. Recent studies have shown the successful use of vitamin A and its synthetic analogues (together known as retinoids) for this purpose^{1,2}. We have shown that both natural and synthetic retinoids (retinyl acetate and retinyl methyl ether, respectively) markedly inhibit mammary carcinogenesis induced in the Sprague-Dawley rat by 7,12-dimethylbenz[*a*]anthracene (DMBA) (refs 3, 4). Although the DMBA model has contributed significantly to the understanding of mammary carcinogenesis, particularly hormone responsiveness, the model is less than ideal in that the tumours only rarely invade surrounding tissues and do not metastasise. A new mammary carcinogenesis model, first described by Gullino *et al.*⁵, using intravenous injection of *N*-methyl-*N*-nitrosourea (NMU) in the Buffalo rat, satisfies the deficiencies of the DMBA model in that cancers are exclusively localised in the mammary gland, are hormone dependent, show metastasis to other organs, are transplantable, and result in hypercalcaemia. NMU also causes a high incidence of mammary cancers in female Sprague-Dawley and Fischer-344 rats⁶, but the biological characteristics of the cancers in these strains have not been described in detail. But, our preliminary studies using Sprague-Dawley rats indicated that NMU-induced mammary cancers are highly aggressive and similar histologically to those induced in the Buffalo rat. The NMU model of mammary cancer thus resembles the human disease more closely than the DMBA model. We describe here a study of the effect of a retinyl acetate on mammary carcinogenesis induced by NMU, using a modification of the procedure reported by Gullino *et al.*⁵. We show that retinyl acetate inhibits carcinogenesis in this system.

Female rats of the Sprague-Dawley strain were obtained when 42 days of age. All the animals initially received stock diet (Wayne Lab Meal) and tap water *ad libitum*. NMU was dissolved in physiological saline (20 mg ml⁻¹) which had been adjusted to pH 5.0 with a few drops of 3% acetic acid. Rats (50-d-old; average weight 150 g) were injected intravenously with NMU at 5.0, 2.5, or 1.25 mg per 100 g of body weight (Table 1). Control animals received saline alone. Each NMU-treated and control rat received a second injection 7 days later. Three days after the second injection, all rats were placed on the diets indicated in Table 1 for the remainder of the experiment. Retinyl acetate was blended into the stock diet in the form of stable gelatinised beadlets at a concentration of 250 mg

retinyl acetate per kg diet; rats in groups 1, 3, 5, and 7 received the control gelatinised beadlet material without retinoid (placebo beadlets). All rats were palpated twice each week for detection of mammary tumours, and were also weighed twice each week and observed daily for evidence of retinoid toxicity. At 175 days all animals were sacrificed and the mammary tumours removed and processed for histological classification. More than 75% of the tumours (Table 1) were adenocarcinomas, which were highly aggressive, contained many mitotic figures, and were invasive to the pectoral muscle. Metastasis to the kidney was found in one rat in group 5. A few benign tumours were also seen, and these were either adenomas or fibroadenomas.

No mammary tumours developed in animals which received no carcinogen. Animals receiving the high dose of NMU and fed placebo diet (group 3) had a mammary cancer incidence of 83% (Table 1), while animals treated with this dose of carcinogen and fed retinyl acetate had a mammary cancer incidence of only 50% (group 4). A similar reduction in the incidence of mammary cancer was found in rats receiving retinyl acetate and the intermediate dose of NMU (group 6 compared with group 5). No mammary cancer developed in those rats receiving the low dose of NMU and retinyl acetate (group 8), while the animals treated with this dose of NMU and placebo diet (group 7), had a 20% incidence of cancer. Although this incidence of mammary cancer in group 7 might be considered relatively low, it is more than twice that found in the total population of women at risk in England or the United States⁸.

In addition to reducing the incidence of mammary cancer, retinyl acetate also caused a marked reduction in the total number of cancers, as well as benign tumours (Table 1). Furthermore, the administration of retinyl acetate greatly prolonged the latency period of mammary cancer at all three doses of NMU. As shown in Fig. 1, the time of appearance of the first palpable cancer in rats treated with the high dose of NMU was doubled by the administration of retinyl acetate. With the low dose of carcinogen, the first palpable cancer appeared at 107 days, whereas animals fed retinyl acetate after the low dose of NMU were free of mammary cancer on termination of the experiment at 175 days.

The mechanism by which retinoids, both natural and synthetic, protect against mammary carcinogenesis is unknown. Here, cancer prevention was not due to an inhibition of initiation of cancer, since feeding of retinyl acetate was not begun until after all free carcinogen had disappeared. The half life of NMU at body pH is only a few hours⁷. Moreover, inhibition of carcinogenesis did not result from some general toxic effect of retinyl acetate, as 'hypervitaminosis A' (ref. 8) was not observed. At necropsy,

Table 1 Effect of retinyl acetate on mammary carcinogenesis

Group	NMU	Retinoid	No. of rats with cancer	Incidence of mammary cancer (%)	Total no. of tumours	
			Total no. of rats		Cancer	Benign
1	None	Placebo	0/30	0	0	0
2	None	Retinyl acetate	0/30	0	0	0
3	High dose	Placebo	25/30	83	61	17
4	High dose	Retinyl acetate	15/30	50*	25*	4*
5	Intermediate dose	Placebo	12/29	41	16	3
6	Intermediate dose	Retinyl acetate	7/30	23*	11*	1
7	Low dose	Placebo	6/30	20	7	2
8	Low dose	Retinyl acetate	0/30	0*	0*	0

Female Sprague-Dawley rats received either saline or NMU in saline (5.0, 2.5, or 1.25 mg per 100 g body weight) by i.v. injection at 50 d of age. A second injection was administered one week later. The animals were placed on either retinyl acetate (250 mg per kg of diet) or placebo beadlets 3 d after the second injection and sacrificed 175 d after the initial NMU injection.

*Significantly different from respective placebo control group, $P < 0.05$ or better.

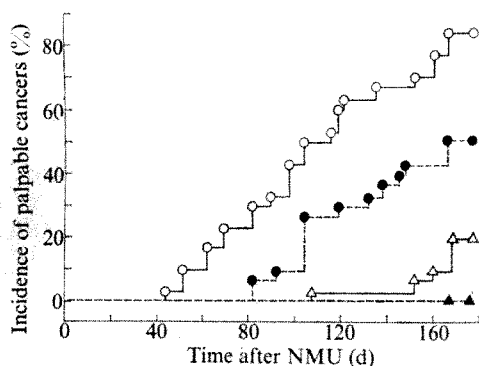


Fig. 1 Effect of retinyl acetate on the time of appearance of palpable mammary cancers which were confirmed histologically at necropsy. Rats were placed on retinyl acetate or placebo diet 3 d after the second i.v. injection of either 5.0 mg or 1.25 mg NMU per 100 g body weight. The rats were palpated for mammary tumours twice weekly for the duration of the experiment. Treatments were: ○, high dose NMU, placebo diet; ●, high dose NMU, retinyl acetate diet; △, low dose NMU, placebo diet; ▲, low dose NMU, retinyl acetate diet.

body weight and liver histology of rats fed retinyl acetate were essentially identical to those fed placebo diet. Vaginal smears from rats fed retinyl acetate revealed normal oestrous cycles, suggesting that metabolism of ovarian steroids was not significantly altered. Although the fundamental effects of retinoids in maintenance of normal epithelial cell differentiation (even in tissues exposed to carcinogens *in vitro*^{9,10}) suggest that this process may be the mechanism of inhibition of carcinogenesis^{1,2}, other effects on the endocrine or immune system cannot be ruled out at the present time.

The present data demonstrate the efficacy of retinyl acetate in prevention of experimental mammary cancer. Although retinoid toxicity was not seen in these studies, the potential toxicity of retinyl esters, such as retinyl acetate or retinyl palmitate^{8,11}, makes the development of new synthetic retinoids, with greater activity and lesser toxicity, of significant importance.

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Synthetic polyamines added to cultures containing bovine sera reversibly inhibit *in vitro* parameters of immunity

POLYAMINES are present in high concentration in foetal¹ and neoplastic tissue² and seminal fluid³—all representing antigenic challenges which usually fail to elicit the appropriate immune response. This has prompted us to investigate the influence of polyamines on *in vitro* parameters of immunity. We report here that micromolar quantities of spermine and spermidine reversibly inhibit three responses of primary cultures of murine spleen cells. These are (1) DNA synthetic response stimulated by phytohaemagglutinin (PHA), pokeweed mitogen (PWM), concanavalin A (con A) or bacterial lipopolysaccharide (LPS); (2) mixed lymphocyte response (MLC) stimulated by irradiated allogeneic cells; and (3) the induction but not the expression of the cytolytic lymphocyte response. The inhibition does not seem to be due to toxicity but occurs only in the presence of calf or foetal calf sera.

When micromolar quantities of spermine and spermidine

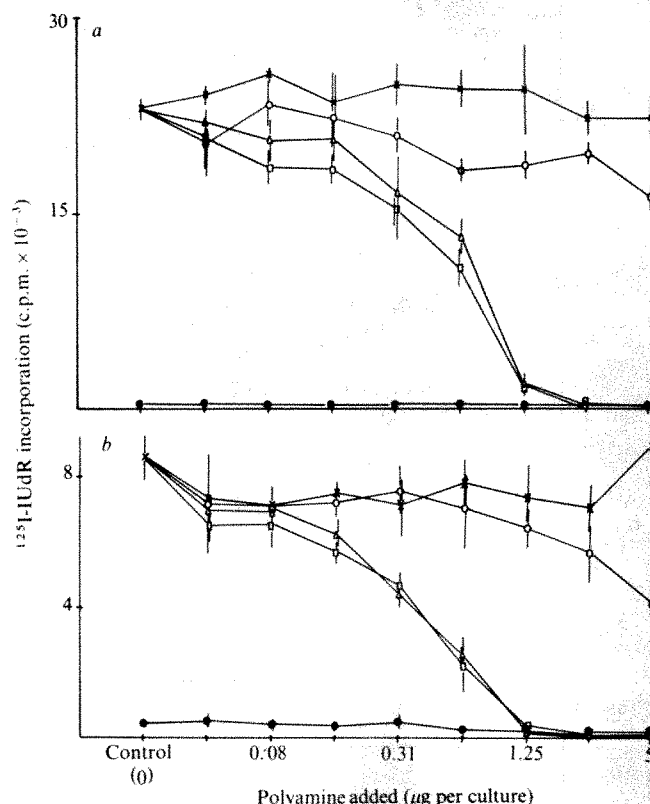


Fig. 1 Effect of polyamines on lymphocyte mitogen responses. 5×10^5 BALB/c spleen cells were cultured in flat bottom Falcon microtest II plates in a volume of 200 μ l in RPMI 1640 medium with 5% FCS which contained nil or a, 0.25 μ g con A (Pharmacia) or b, 1.0 μ g *E. coli* 055:B5 lipopolysaccharide (Difco). The indicated amount of polyamine (obtained from Calbiochem as cadaverine dihydrochloride, putrescine dihydrochloride, spermidine trihydrochloride and spermine tetrahydrochloride dissolved in serum-free medium) was added in 50 μ l making the final volume 250 μ l. Cultures were pulsed with 0.1 μ Ci ^{125} I-IUDR (250 μ Ci μ g⁻¹) (New England Nuclear) after 44 h of culture and cells were collected 18 h later using a multiple automated sample collector (Otto Hiller, Madison, Wisconsin). Samples were counted in a Packard automatic gamma spectrometer. The mean and standard deviations of quadruplicate cultures are shown. Polyamines tested were putrescine ($\text{NH}_2(\text{CH}_2)_4\text{NH}_2$) (×), cadaverine ($\text{NH}_2(\text{CH}_2)_5\text{NH}_2$) (○), spermine ($\text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}(\text{CH}_2)_3\text{NH}_2$) (□) and spermidine ($\text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}_2$) (△). All cultures which did not contain mitogen (●) but did contain varying concentrations of polyamine gave essentially the same low incorporation rate as shown by this representative plot.

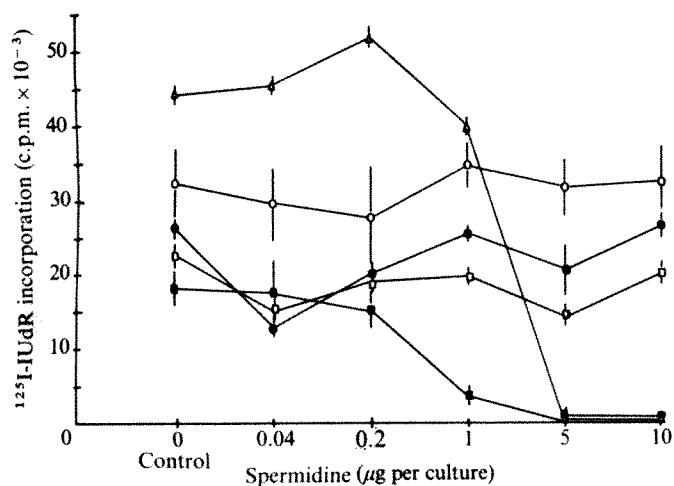


Fig. 2 Serum requirements for spermidine inhibition of mitogen responsiveness. Spleen cells were cultured, additions made, and DNA synthesis measured as described in the legend to Fig. 1, except that the amount of mitogen per culture was 0.5 μ g con A. FCS (▲), calf serum (■) obtained from calves 3–6 months of age, and human serum (○) were present at a final concentration of 4% and mouse serum (●) at 0.4%. Cells cultured in serum-free medium are represented by (□). Essentially identical curves were obtained for PHA, PWM and LPS.

were added to cultured murine spleen cells stimulated with LPS or con A, the DNA synthetic response was inhibited. Identical doses of putrescine and cadaverine had no effect (Fig. 1). Similar results were obtained for PWM and PHA stimulated cultures. Larger doses of polyamine were required to suppress the response to PHA or con A than to suppress that to LPS and PWM. The inhibition could be demonstrated with either calf or foetal calf serum (FCS) in the cultures. In parallel cultures with human or mouse serum, or with no serum, there was no inhibition in spite of the presence of up to 10 μ g per well of spermine and spermidine (Fig. 2).

When FCS was titrated into cultures containing a constant 4% human serum, polyamines inhibited LPS and con A stimulated mitogenesis when the FCS concentration reached 0.5–1.0%. This suggests that some component of FCS is essential for the inhibition.

We next tested whether the observed inhibition was due to toxicity. First we examined the effect of spermine added at different times after inception of standard cultures

stimulated with either con A or LPS. When spermine was added up to 29 h after inception of culture, it could completely inhibit the LPS response. To inhibit the con A completely, spermine had to be added within 22 h of culture inception. The later the polyamine was added, the more was required to obtain the same level of inhibition. This argues against a simple mechanism of acute toxicity but is consistent with an effect on some early event in the differentiation of mitogen-stimulated lymphoid cells.

To investigate the reversibility of the inhibition, we preincubated murine spleen cells (2.5×10^7 in 50 ml of medium RPMI 1640 and a final concentration of 2% FCS with no additions or with spermine ($10 \mu\text{g ml}^{-1}$) or spermidine ($10 \mu\text{g ml}^{-1}$) for either 24, 48 or 72 h. Cultures were then collected, washed three times and subcultured in the microtest II system (see legend of Fig. 1) where they were stimulated with PHA, PWM, con A, or LPS. The responses were compared with those of fresh murine spleen cells obtained each day and cultured in parallel. Control cells, preincubated for 24 h without polyamine, responded well (although less than did fresh cells) to each mitogen when tested in subculture: cells preincubated for 24 h with supra-inhibitory doses of polyamine responded as well as did preincubated control cells in culture.

Cells cultured with and without polyamine for 48 h responded less well to T cell mitogens in general, but there was no significant difference between those preincubated without polyamine and those preincubated in spermidine. By this time, however, all preincubated cells have lost their ability to respond to LPS and PWM and the level of radiolabel incorporation by preincubated cells subcultured without stimulus has increased significantly.

After 3 d of preincubation no cells responded to the mitogens when subcultured. There was no significant difference in the recovery of total viable cells preincubated in spermidine compared to control medium.

The fact that spermine or spermidine at 5–10 μ g per well completely inhibited the response to mitogen when added at the inception of culture (Fig. 1) or 22 h later, but not after preincubation in either polyamine for 24 h suggests that a toxic principle was not responsible for the lack of iododeoxyuridine (IUdR) incorporation in the subcultures. It is consistent with polyamine affecting some event in stimulated cells rather than having a nonspecific deleterious effect on quiescent cells.

Polyamines also inhibit the ability of murine lymphocytes to recognise irradiated allogeneic cells (Table 1). Furthermore, the ability of cells in a mixed lymphocyte culture to

Table 1 Influence of synthetic polyamines on murine mixed lymphocyte and cell-mediated cytotoxicity responses of CBA spleen cells stimulated by irradiated DBA/2 spleen cells

	Spermine added (μ g)	^{125}I -IUdR incorporated (c.p.m.) $\times 10^3$		
		Day 4	Day 5	Day 6
5×10^5 CBA + 5×10^5 DBA	nil	26.0 ± 5.00	64.6 ± 2.70	30.9 ± 3.06
	0.31	15.4 ± 3.40	40.5 ± 9.19	32.7 ± 6.14
	1.25	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.12
	5	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.02
	20	0.1 ± 0.04	0.3 ± 0.13	0.5 ± 0.06
5×10^5 DBA + nil	nil	0.3 ± 0.12	1.6 ± 1.7	0.9 ± 0.54
			% Chromium released	
	nil		56.3	
	0.31		59.1	
	1.25		7.2	
	5.0		1.8	
	20.0		0	

^{125}I -IUdR incorporation data are means of four replicate cultures ± 1 s.d. Cells were collected from identical prepared cultures on days 4–6 with the peak control response occurring on day 5. Note that as little as 310 ng of spermine significantly suppressed the response while 1.2 μ g or more of spermine completely suppressed the response. Where polyamine was present only during the expression phase of the cytotoxic response the percentage of chromium release was in excess of 45%. Addition of as much as 20 μ g of polyamine to chromated P-815 target cells alone showed only a 1–2% release of chromium indicating that polyamine *per se* is not acutely toxic to cells known for their fragility.

mount a non-complement-dependent cytolytic response is impaired if polyamines are present from the inception of the culture. Polyamines present only during the chromium release phase of culture do not alter the response nor do they increase the spontaneous release of chromium from the target cells.

These results indicate that certain polyamines have a reversible, suppressive effect on *in vitro* parameters of immunity; a finding of interest for several reasons. The inhibitory property of the product of bovine serum-polyamine interaction may explain why there is only limited DNA synthesis or antibody formation in most studies of *in vitro* immune responses. Lymphocytes contain nanomolar quantities of spermine⁴ and in culture they are normally supplemented with bovine serum. In most *in vitro* systems 60–80% of cells die and probably sufficient polyamine could be released into the medium to react with the bovine serum, form the inhibitor and thus render the response self-limiting.

Spermine and spermidine themselves are not inhibitory; they must either be converted into some other active moiety by the bovine serum or alternatively cause the bovine serum to release an unrelated active molecule.

The possibility that the observations described here were due to toxicity was suggested by a report that a specific amine oxidase of ruminant sera can convert spermine or spermidine to aminoaldehydes toxic to cell cultures⁵. Also, Alarcon found acrolein ($\text{CH}_2\text{CH:CHO}$) in mixtures of bovine sera and polyamine, and wondered whether this might account for the toxicity of the mixtures because acrolein is toxic⁶. We do not believe, however, that either possibility applies to our observations. The strongest evidence against toxicity is the finding that cells preincubated for 24–48 h in supra-inhibitory doses of spermidine later responded to mitogen stimulation as well as did cells preincubated in control medium. It seems unlikely that fixatives such as acrolein or aminoaldehyde would reversibly fix the lymphocyte surface.

Cells involved in the immune response seem to be particularly susceptible to polyamines compared with fibroblasts, for example. Polyamines are widely distributed in mammalian tissues⁷. They are also present in very high concentration in foetal and neoplastic tissues; both of which are known to possess specific antigens but still avoid immunological rejection^{1,8,9}. We postulate that the successful subversion of the host immune system in those individuals bearing tumours or outbred foetuses is mediated by polyamine or a polyamine interaction product. It is conceivable that the product of the interaction of polyamine and bovine serum could inhibit immune reactivity in a general way, and thus represent a natural immunoregulatory agent.

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Identification of a thymic inhibitor ('chalone') of lymphocyte transformation as a spermine complex

CHALONES are defined as endogenous, tissue-specific but species nonspecific inhibitors of cell proliferation. Their potential use in the control of neoplasia is well recognised^{1–3}. Circumstantial evidence for the existence of a lymphocyte chalone has been produced^{4–8}, but neither its chemical nature nor its molecular weight have been satisfactorily ascertained. During our search for a lymphocyte chalone, an inhibitor of lymphocyte transformation has been reproducibly isolated from porcine thymic tissue. We report here that though the inhibitor was non-dialysable, its active moiety was identified biologically and chemically as spermine, molecular weight only 202. This accords with the formation in thymic extracts of a tightly bound spermine complex. As the complex displayed some tissue specificity it is possible that an unidentified tissue-specific material acts as a carrier for spermine. In relatively crude extracts of tissues it is probable that spermine-elicited inhibition swamps any authentic chalone activity.

Extracts of thymic tissue were prepared and fractionated by the method of Kiger *et al.*⁵. Briefly, ethanol was added (4 °C) to an aqueous thymic extract (fraction TAE) to a concentration of 42% by vol. The soluble portion (fraction T₁) was dialysed (Visking tubing) against distilled water and subjected to ion-exchange separation on DEAE A-50 Sephadex (Pharmacia). A fraction analogous to (F₂–F₆) of Kiger *et al.*⁵ was obtained, and thoroughly dialysed against distilled water. Fractions were freeze-dried for storage at –20 °C. The most highly purified (F₂–F₆) sub-fraction contained a mixture of material with a molecular weight ranging from 50,000 down to 800 as defined by P-60 and P-2 (Biorad) gel filtration.

Table 1 Comparison of inhibitory doses (ID) of thymic extracts and polyamines on PHA-stimulated lymphocyte, fibroblast and granulocyte uptake of ³HTdR (DNA synthesis) in medium containing foetal calf serum

Fraction	Lymphocytes ID ₅₀	Fibroblasts ID ₅₀	Granulocytes ID ₅₀
TAE	275 µg ml ⁻¹	> 1,000 µg ml ⁻¹	> 1,000 µg ml ⁻¹
T ₁	210 µg ml ⁻¹	1,000 µg ml ⁻¹	1,000 µg ml ⁻¹
(F ₂ –F ₆)	100 µg ml ⁻¹	500 µg ml ⁻¹	500 µg ml ⁻¹
(F ₂ –F ₆) polyamines	20 µg ml ⁻¹	20 µg ml ⁻¹	25 µg ml ⁻¹
Spermine	12 µM	12 µM	15 µM
Spermidine	36 µM	36 µM	n.t.
Putrescine	> 160 µM	n.t.	n.t.

Triplicate 1.0 ml cultures of 5 × 10⁶ young male rat (Sprague-Dawley strain) thymic lymphocytes were incubated with 15 µg of purified PHA (Wellcome) in 75 × 10 mm tubes (Falcon) for 62 h, and uptake from 1.0 µCi of ³HTdR (2 Ci mmol⁻¹) or ³HUdR (10.3 Ci mmol⁻¹, Radiochemical Centre) was measured for the last 16 h of incubation. Hamster lung fibroblasts (Yerganian cell line) were established in Leighton tubes (1.0 ml triplicate cultures) and uptake of 1.0 µCi was measured over one generation of cells (16 h). Rat granulocytes were assayed by the method of Paukovits¹⁷. Medium RPMI 1640 (Flow) containing antibiotics, 2 mM L-glutamine, 16 mM NaHCO₃ (buffer) and 12.5% foetal calf serum (Rehatuin, Armour) were used for all cultures. The same batch of serum was used throughout. Completed media containing thymic extracts or polyamine were incubated for 1–2 h before adding cells. Reagents were sterilised by filtration through Millipore 0.22 µm membranes. Cultures were incubated at 37 °C in an atmosphere of 5% CO₂ in air. Results were calculated from a dose-response curve plotted for the mean d.p.m. (background subtracted) of triplicate cultures. Standard deviations were less than 12% of the mean. Relative uptake of ³HUdR was similar to that for ³HTdR and so is not considered in the Table. Measurement of incorporation of radio labels in TCA-precipitable material of washed collected cells in PCS scintillator (Searle) was made using a Beckman spectrophotometer. n.t., Not tested.

Thymic fractions TAE, T₁ and (F₂-F₆) inhibited DNA synthesis (measured by uptake of ³HTdR) and RNA synthesis (uptake of ³HUdR) equally, in phytohaemagglutinin (PHA)-stimulated rat thymic lymphocytes. In contrast, the inhibition was significantly less when assayed against fibroblast or granulocyte proliferation. Representative results are summarised in Table 1. In addition, administration of fraction (F₂-F₆) to CBA recipient mice for full-thickness WH strain mouse tail-skin allografts at a dosage of 7.0 mg per mouse on days -1, 1, 3 and 6 after grafting, produced significant ($P=4.75$ for 12 d.f.) but not dramatic prolongation of graft survival from 10.14 ± 0.28 (s.d.) to 12.86 ± 0.51 d.

Thus the extract (F₂-F₆) was shown to exhibit chalone-like activity both *in vitro* and *in vivo*. A remarkable finding was that all the above fractions completely lost inhibitory activity when instead of foetal calf serum, human serum (adult or foetal-cord) was used to supplement cultures. Results using fraction (F₂-F₆) are shown in Fig. 1. Byrd *et al.*⁹ have reported that potent inhibition of lymphocyte transformation was evoked by the polyamines spermine and spermidine, and that the phenomenon was similarly dependent on the presence of bovine serum. We have observed similar effects and shown fibroblasts and granulocytes to be equally susceptible (Table 1); putrescine was not an inhibitor. Moreover, injection of 1.0-mg doses of spermidine (Sigma) in the schedule described above caused a somewhat longer retention of skin grafts than previously obtained with fraction (F₂-F₆).

Experiments still in progress indicate that the inhibitory properties of the polyamines are closely linked to plasma amine oxidase activity, which is abundant in ruminant sera but shows little activity in human sera. If this is so, the aldehydes produced by the enzymic oxidative deamination of spermine and spermidine must be exceedingly potent inhibitors. Consequently, our investigations were continued to explore the possibility of the thymic fractions containing

Fig. 1 Inhibitory activity of thymic fraction (F₂-F₆) on the uptake of ³HTdR by PHA-transformed lymphocytes in foetal calf serum (●) and absence of activity in human serum (○). Culture details are presented in Table 1. Each point represents the mean d.p.m. of triplicate cultures $\pm$ s.d. (background subtracted).

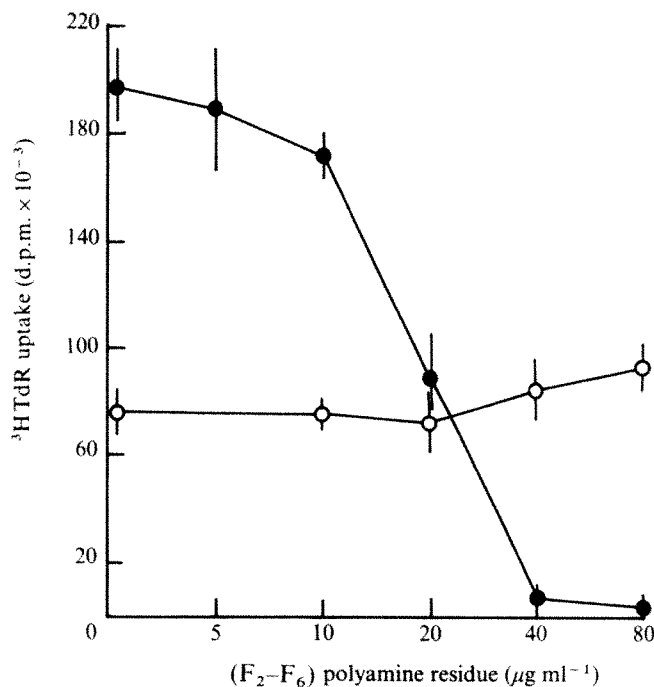
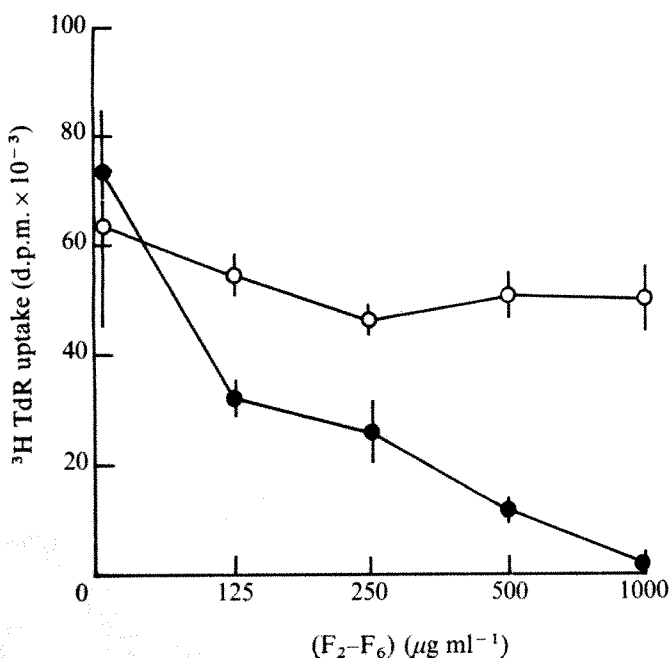


Fig. 2 Inhibitory activity of polyamine residue separated from thymic fraction (F₂-F₆) on uptake of ³HTdR by PHA-transformed lymphocytes in foetal calf serum (●) and in human serum (○). Culture details are presented in Table 1. Each point represents the mean d.p.m. of triplicate cultures $\pm$ s.d. (background subtracted).

polyamines. Fraction (F₂-F₆) was subjected to the polyamine extraction procedure of Inoue and Mizutani¹⁰. The hydrolysed residue obtained potentially inhibited lymphocyte transformation in foetal calf serum ($ID_{50}=20 \mu\text{g ml}^{-1}$) but not in human serum (Fig. 2). Analysis by gas-liquid chromatography (GLC) of the trifluoroacetylated residue using the method of McGregor *et al.*¹¹ confirmed that the residue contained roughly 20% spermine together with a trace of spermidine. On the basis of its spermine content, the residue had an ID_{50} of about $4.0 \mu\text{g ml}^{-1}$, reasonably close to that of authentic spermine. The loss of apparent tissue specificity on extraction of spermine from fraction (F₂-F₆) suggests that the spermine may exist in (F₂-F₆) as a tightly bound complex, with the carrier conferring the specificity. In addition, since both spermine and spermidine are present in normal tissues¹², the carrier itself showed a preference for spermine.

Finally, a series of experiments using ¹⁴C-spermine tetrahydrochloride were conducted, to study the extent of dialysis of spermine from the thymic fractions and from other materials. The essential finding was that 0.6% to 3.5% of spermine initially present could survive the extraction procedure (full details will be published elsewhere). Available data on polyamine concentrations in tissues¹³ show that this is quite sufficient to account for the amount isolated and identified in this work.

These results emphasise the difficulties associated with the isolation of an authentic lymphocyte chalone, but by no means rule out its existence. Polyamines have been implicated in growth processes¹⁴⁻¹⁶ and increased cellular levels are associated with optimal PHA-induced transformation of lymphocytes¹⁷. The activation of spermine, possibly with an amine oxidase, may thus represent a sensitive physiological control mechanism. The possibility that this mechanism is mediated by a larger, specific carrier molecule is not discounted.

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Calcium ion produces graded changes in permeability of membrane channels in cell junction

THE permeability of the membrane channels in *Chironomus* salivary gland cell junction depends on the cytoplasmic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) (refs 1, 2). At the normal $[\text{Ca}^{2+}]_i$, $< 10^{-7}$ M, the channels are highly permeable to a wide range of molecular sizes; the upper size limit for peptide molecules is about 1,200–1,900 molecular weight (MW) (refs 3,4). When the $[\text{Ca}^{2+}]_i$ is elevated above 5×10^{-5} M in the junctional locale, the permeability falls drastically for all molecular species including the small inorganic ions^{2,5,6}. With elevations ranging up to about 5×10^{-5} M the channel permeability is reduced for the 330-MW fluorescein molecule with little or no detectable reduction of electrical coupling⁷. This suggested the interesting possibility that in this range the permeability change may be selective, that is, the permeability may fall for the larger molecular species but not for the small inorganic molecules carrying the electrical current. An alternative—that a fraction of the junctional channels closed unselectively (that is, completely)—could not be excluded, however, because the sensitivities of the methods for discriminating changes in the junctional permeabilities of the large and small molecular species were not equivalent; the measurements of electrical coupling, while quite sensitive to changes in junctional permeability in the low range of electrical coupling, are relatively insensitive at high levels of coupling⁷. Here we investigate this point by testing junctional permeability with fluorescent molecules of various sizes. In each test we use a pair of molecular species and compare their transit times during simultaneous diffusion in the same direction across the junction. If and only if the non-selective mechanism governs the Ca^{2+} -mediated permeability reduction, the transit for both species should be equally retarded, irrespective of size or structural differences between the probe molecules. We show that, at low $[\text{Ca}^{2+}]_i$

elevation, the junctional permeability reduction is indeed selective and that the molecular size limit for junctional channel permeation changes in a graded manner.

A series of seven water-soluble fluorescent-labelled molecules ranging from 380 to 1,158 MW were used as permeability probes of the junction of *Chironomus* salivary gland cells (mid-fourth instar). These tracer molecules permeate the junctional membrane but not significantly the non-junctional membrane³ and, at the $[\text{Ca}^{2+}]_i$ used, their Ca binding is negligible and their (paper) electrophoretic mobility is unchanged. The tracer molecules were injected into the cells using micropipettes and a pneumatic pressure system². Permeabilities were tested by pair comparisons; each pair of tracers to be studied (Table 1) was injected as a mixture. In every pair, one tracer had a yellow-green fluorescent label of fluorescein isothiocyanate (FITC) or dansyl (DANS), and the other, a red fluorescent label of lissamine rhodamine B (LRB). Thus, the members in the pairs were easily distinguishable in the cells by means of appropriate barrier filters. The concentration ratio for a given pair was approximately constant and the fluorescence intensities were roughly matched. Only changes in transit time are of interest, however, so differences in absolute concentration and in detectability were of no consequence (so long as source concentration $\gg$ threshold for detection). The intracellular and transjunctional spread of the fluorescent tracers was observed and photographed directly in a darkfield microscope and/or scanned and videotaped through an image intensifier-television system². The tracers moved through the cytoplasm and the junction with velocities inversely related to their molecular weights; their arrival at the junction was marked by an abrupt fall in velocity³. Junctional permeabilities were measured as transit time, that is, the interval between the moment the tracer arrives at the junction and the moment it appears in the adjacent cell. Junctional transit speed in elevated $[\text{Ca}^{2+}]_i$ will be given relative to speed in Ca-free controls (determined at the same concentration ratio for a given pair); the relative transit speed (v), then, is defined as the ratio of the mean transit time of controls to transit time in the presence of Ca^{2+} . The experiments were carried out at room temperature, 22–25 °C.

Ca^{2+} buffered with citrate or mixed with ethyleneglycol-bis (β -aminoethyl ether)- N - N' -tetraacetic acid (EGTA) was injected together with the tracers or, in some cases, separately before the tracer injection. The free Ca^{2+} concentration in the injection solutions ranged from 10^{-4} to 10^{-2} M: this concentration had to be so high because Ca^{2+} is rapidly sequestered inside the cells and its concentration falls off precipitously with distance from the injection point. By the time the injected Ca^{2+} reaches the junction, some 50 μm away from our standard injection point (about cell centre), its concentration has fallen several orders of magnitude⁸. With the quantities and rates of the present Ca^{2+} injections, we expect, on the basis of earlier work in which the spatial distribution of $[\text{Ca}^{2+}]_i$ was determined using aequorin², that the $[\text{Ca}^{2+}]_i$ elevations in the junctional locale were in the range of 10^{-7} to 10^{-5} M.

The basic result was that, on elevation of $[\text{Ca}^{2+}]_i$ in this range, the transjunctional flow of the larger molecules was

Table 1 Tracer pairs

Pair no.		Molecular weight		Molecular weight
I	LRB (Leu) ₃ (Glu) ₂ OH	1,158	/DANS (Leu) ₃ (Glu) ₂ OH	849
II	LRB (Leu) ₃ (Glu) ₂ OH	1,158	/FITC (Glu) ₃ OH	794
III	LRB (Leu) ₃ (Glu) ₂ OH	1,158	/DANS (Glu) ₃ OH	640
IV	LRB (Leu) ₃ (Glu) ₂ OH	1,158	/DANS (Glu) OH	380
V	LRB (Gly) ₆ OH	901	/DANS (Glu) OH	380
VI	DANS (Leu) ₃ (Glu) ₂ OH	849	/LRB SO ₃ H	559
VII	FITC (Glu) ₃ OH	794	/LRB SO ₃ H	559

LRB, Lissamine rhodamine B; DANS, dansyl (dimethylamino-naphthalene sulphonyl); FITC, fluorescein isothiocyanate; Glu OH, glutamate; Leu OH, leucine; Gly OH, Glycine.

slowed more than that of the smaller ones and in some cases was even sensibly blocked while the small molecules were passing.

With Ca-free control solutions, containing EGTA or citrate, all seven tracer molecules passed through the junction with transit times ranging from a few seconds to about 1 min, depending on size. The transit times were the same as when EGTA or citrate was omitted³. With Ca-containing solutions the junctional transits were retarded, and in almost every test with pairs *III* to *VII* (Table 1) the retardation of the larger molecule (L) exceeded that of the smaller companion (S). Figure 1 presents the complete data for relative junctional transit speeds (v) of tracer pairs *III*, *IV*, *VI* and *VII*. The plots include also those cases with selective block of one molecule in a pair, invariably the larger ($v_L \approx 0$). Drawn in each graph is the line ($v_L/v_S = 1$) that would represent equal retardation of both tracers, namely non-selective retardation of junctional permeability. Nearly all points fall below the line. Application of the two-sample t test to the aggregate data of each graph, to compare the retarded junctional tracer movement with the control tracer movement, shows clearly that for all four tracer pairs the permeability reduction is selective; the confidence levels for $v_L/v_S < 1$ are 0.5, 5, < 0.5, and < 0.1% respectively for the pairs *III*, *IV*, *VI* and *VII*. A smaller number of experiments with *V* gave the same results; although only 6 trials were made, the confidence level was 3%. No selective junctional permeability reduction was found with the two pairs of the four largest molecules, pairs *I* and *II*; within the resolution of the method, the junctional transits of these molecules were equally retarded by $[Ca^{2+}]_i$ elevation. Eventually, at high $[Ca^{2+}]_i$, all seven molecules were blocked.

A significant feature of the results is the occurrence of selective block of junctional transit of the larger molecule in the pairs giving selective permeability reduction (two examples are

Fig. 1 Selective retardation of junctional transit by Ca^{2+} . Plotted are the relative junctional transit speeds (v) modified by Ca^{2+} for the small (S, abscissae) and large members (L, ordinates) of tracer pairs *III*, *IV*, *VI* and *VII*. Each value of v is the ratio of the mean junctional transit time in Ca-free controls to a single measurement of transit time in elevated $[Ca^{2+}]_i$. The subscripts give the molecular weights of the tracers. Each graph presents all data for a given tracer pair, including those of selective block (circled; $v_L = 0$). Squares denote repeated data points, with the multiplicity indicated. The line represents $v_S = v_L$, that is, equal (non-selective) retardation of junctional transit. The resultant confidence levels for $v_L < v_S$ are (*III*) 0.5, (*IV*) 5, (*VI*) < 0.5 and (*VII*) < 0.1%. (The numbers of controls and two other underlying statistics, the ratios of mean transit times (L/S) of the controls $\pm$ s.e. were: (*III*) 5; 1.29 ± 0.27 ; (*IV*) 8; 2.67 ± 0.58 ; (*VI*) 5; 2.36 ± 0.54 ; (*VII*) 11; 1.18 ± 0.16 , respectively.)

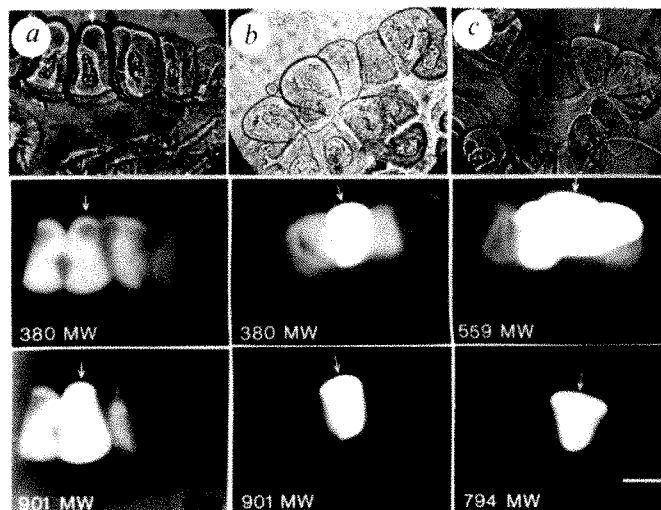
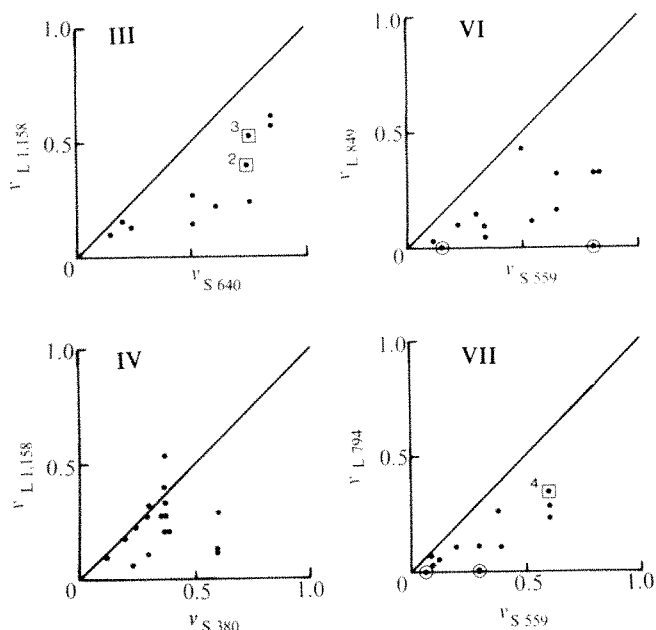


Fig. 2 Selective block of junctional transit. Permeability of junctional membrane channels is probed with pairs of fluorescently-labelled tracers. The fluorescence of one tracer in each pair is yellow-green and the other is red. The pairs are microinjected into a cell (arrow) together with Ca-buffers and the tracer fluorescence is photographed. Each series of photographs gives, from top to bottom, a view of the cells; the intracellular fluorescence of the smaller tracer molecule; that of the larger molecule (set apart by excitation and barrier filters.) The molecular weights on the photographs identify the tracer pairs. *a*, control; both molecules of the pair have passed from cell to cell. *b* and *c*, the molecules are injected together with Ca^{2+} ; passage of the larger molecule is blocked. The Ca-citrate ratios were: *a*, 0; *b*, 0.8; *c*, 1.0; citrate_{total}, 0.03 M. The photographs of the blocked tracers (*b* and *c*) were taken 5 min after those of their respective smaller companions; the interval injection-to-photography was in *b* and *c* 35-fold the control transit time. In *a* the photograph of the larger tracer was taken before that of the smaller tracer; the interval injection-to-photography for the larger tracer was 15-fold the control transit time. Calibration, 100 μ m.

illustrated in Fig. 2). This shows that the molecular weight limit for junctional permeation decreases on $[Ca^{2+}]_i$ elevation, and decreases in a graded fashion. If a stepwise decrease in molecular limit for junctional permeation is assumed, then, with the resolution afforded by the present probe repertoire, one may admit at least one gradation: one between 1,158 and 640 MW (or between 794 and 559). If we extrapolate now to the earlier result showing a dissociation between the junctional passage of the molecule of 330-MW (fluorescein) and electrical coupling, two further steps seem likely: one between 330 MW and the size of the smallest inorganic ion; and a third where even the latter ion is barred from junctional passage as the $[Ca^{2+}]_i$ concentration at the junction rises above about 5×10^{-5} M (refs 2,6). The actual grading may, of course, be finer and charge properties of the molecules may be additional contributing factors.

In seeking a controlling variable for the grading in junctional permeability, one is drawn to $[Ca^{2+}]_i$. Not only is this variable continuous, but at extreme values (10^{-7} M and $\sim 5 \times 10^{-5}$ M) it is associated with the extremes of junctional permeability, and an intermediate range is associated with intermediate permeabilities.

Seen in terms of the idea that the permeable junction is made of unit channels^{4,9-11}, the gradual decrease of the molecular size limit may be attributed to a gradual diminution of effective channel size on $[Ca^{2+}]_i$ elevation. The junction would behave like a sieve in which the unit mesh can be controlled by Ca^{2+} . Alternatively, the junction might contain channels of different size and Ca sensitivity. Here the result would be accounted for by an all-or-none closure of channels with Ca^{2+} sensitivities directly related to channel size.

As to the mechanism whereby Ca^{2+} affects the channels, it is plausible that this ion binds to junctional membrane causing a change in the channel's fixed charge or molecular conformation that reduces its effective size (by a decrease in the size of the membrane channels or by their misalignment on the two

membranes)^{4,7}. There is electron microscopic and spectroscopic evidence for Ca deposits at junctional membranes¹²⁻¹⁴ and for changes in size and packing of membrane particles in 'gap' junction in conditions where $[Ca^{2+}]_i$ is expected to have risen¹⁵.

The graded control of junctional permeability by Ca^{2+} offers in principle a powerful mechanism for physiological regulation of intercellular communication; it provides a means for selective transmission of intercellular molecular signals. A case in point may be in embryonic development where, as proposed earlier¹⁶, selective uncoupling of molecular signals could provide an economical means for producing stable cellular differentiations. In fact, for early embryos, there is evidence that fluorescein may not pass between electrically coupled cells¹⁷⁻¹⁹. If this phenomenon turns out to be due to a truly selective restriction of permeability (rather than due to a non-selective reduction in the number of channels; see above), it will be interesting to see whether the $[Ca^{2+}]_i$ is elevated at this developmental stage.

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Selective inhibition of thromboxane A_2 biosynthesis in blood platelets

SAMUELSSON'S group^{1,2} showed that human platelets convert arachidonic acid and prostaglandin (PG) endoperoxides³ to thromboxane A_2 (TXA₂). TXA₂ strongly aggregates platelets¹ and contracts vascular strips^{1,4,5}. Vane's group^{5,6} has identified TXA₂ synthetase in platelet microsomes—this enzyme generates TXA₂ from PG endoperoxides. Aspirin-like drugs inhibit generation of PGs (refs 7, 8) and TXA₂ (ref. 9) in platelets at the early stage of cyclooxygenation of arachidonic acid^{10,11}. It is through this activity that aspirin, indomethacin, naproxen and other acidic anti-inflammatory drugs are anti-aggregatory^{11,12}—they have little or no effect on generation of TXA₂ from PG endoperoxides⁶. We report here that the non-acidic anti-inflammatory compound 2-isopropyl-3-nicotinyl-indole (L 8027) (refs 13, 14) is a selective TXA₂ synthetase inhibitor in platelets and a potent anti-aggregatory agent.

The inhibitory effect of L 8027 on the PG synthetase system was evaluated in bovine (BSVM) and ram (RSVM) seminal vesicle microsomal preparations. Fresh BSVM (refs 15, 16) or lyophilised RSVM (ref. 17) (2-3 mg ml⁻¹) were incubated with sodium arachidonate (33 μ M) in 66 mM phosphate buffer pH 8.0 for 5 min at 37 °C or at 30 °C, respectively. The incubation mixture with BSVM also contained glutathione (166 μ M) and hydroquinone (45 μ M). L 8027 (1-40 μ M) was pre-

incubated with the microsomal preparations for 6 min before the substrate was added. The activity of cyclo-oxygenase in RSVM was evaluated by polarographic measurement of the oxygen uptake¹⁸. The activity of PG synthetase system in BSVM and in RSVM was evaluated by bioassay of the PG-like product¹⁶. In BSVM 80-90% of the bioassayed PG-like activity was PGE₂ (ref. 13). The biologically active products which were formed from arachidonic acid by RSVM in the absence of cofactors were not separated by chromatography. The concentrations of L 8027 required to inhibit the enzymic activity by 50% (IC₅₀) were: 5.9 μ M in BSVM, 17 μ M in RSVM (as measured by bioassay) and 13 μ M in RSVM (as measured by oxygen consumption). Thus we found L 8027 to be 60-180 times less potent than indomethacin and 10-30 times more potent than aspirin as a PG synthetase inhibitor. This is in agreement with the data of Deby *et al.*¹³ on the relative anti-PG synthetase potency of L 8027.

TXA₂ synthetase activity in platelet microsomes was evaluated by an indirect method. The PG endoperoxide generating system of RSVM (ref. 3) was coupled⁶ to TXA₂ synthetase of horse platelet microsomes (HPM) (ref. 5). The biological activities of PG endoperoxides and TXA₂ were differentially assayed on a strip of rabbit mesenteric artery which is relaxed by PG endoperoxides and contracted by TXA₂ (ref. 19). Other bioassay organs used were rabbit aortic strip²⁰ and rabbit vena cava¹⁹ (both contracted by TXA₂ and by PG endoperoxides), rat stomach strip²¹ and rat colon²². Rat stomach strip is contracted by PEG₂, PGF_{2 α} , PG endoperoxides and TXA₂ in a decreasing order of potency⁹. Rat colon is not sensitive to TXA₂ and PG endoperoxides (up to 100 ng), but is contracted by PGF_{2 α} and PGE₂. The assay tissues were superfused in cascade²³ with Krebs bicarbonate (3 ml per min, 37 °C), containing a mixture of combined antagonists²⁴ and indomethacin (1 μ g ml⁻¹). In three experiments the concentrations of L 8027 needed for 50% inhibition of TXA₂ synthetase in HPM were 0.25 μ M, 0.3 μ M and 0.8 μ M. Thus L 8027 was 16-52 times more potent as an inhibitor of TXA₂ synthetase in HPM than as inhibitor of cyclooxygenase in RSVM.

In rabbit platelet homogenates L 8027 at concentrations of 0.3-1 μ M also inhibited TXA₂ generation from arachidonic acid (three experiments as in Fig. 1). At the same time L 8027 hardly influenced PG generation by platelet homogenates as shown by contractions of rat colon (Fig. 1).

The most striking inhibitory effect of L 8027 on TXA₂ generation, however, occurred in aggregating platelets. Aggregation of platelets in 1 ml of fresh citrated rabbit platelet-rich plasma (PRP) was monitored in a Chrono-Log aggregometer. Aggregation was induced by sodium arachidonate (AA, 25-200 μ M) or by adenosine diphosphate (ADP, 10 μ M). L 8027 was preincubated with PRP at 37 °C for 6 min before an aggregating agent was added. Sixty seconds later a 100- μ l aliquot of PRP was withdrawn and immediately bioassayed on a strip of rabbit mesenteric artery for TXA₂. In 10 PRP the threshold pro-aggregatory effect of AA (25-70 μ M) was completely abolished by L 8027 at concentrations of 40 pM-10 nM. Anti-aggregatory concentrations of L 8027 mounted up to 0.1-1.0 μ M in parallel to an increase in the pro-aggregatory concentrations of AA (100-200 μ M). These anti-aggregatory concentrations of L 8027 (40 pM to 1 μ M) also dramatically suppressed the release of TXA₂ from platelets by AA. In eight PRP the ADP-induced aggregation was not inhibited by L 8027 at concentrations up to 40 μ M. ADP-induced aggregation was not associated with the detectable release of TXA₂.

The selective inhibition of TXA₂ synthetase by L 8027 in horse platelet microsomes and in rabbit platelet homogenates explains the potent anti-aggregatory effect of L 8027 on rabbit platelets. But, high anti-aggregatory potency of L 8027 against the threshold pro-aggregatory concentrations of arachidonate as well as an instant drop of this potency in parallel to an increase in concentration of the pro-aggregatory agent indicates that anti-TXA₂ synthetase activity of L 8027 is strongly dependent on the substrate concentration. It could be that L

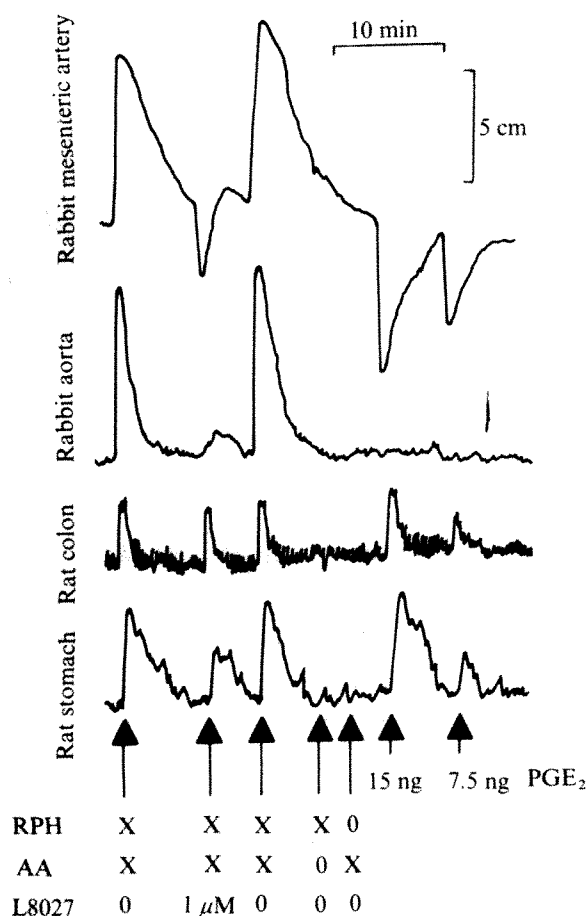


Fig. 1 Bioassay of thromboxane A_2 and prostaglandins generated during an incubation of arachidonic acid (AA) with rabbit platelet homogenate (RPH). Platelets were obtained from 40 ml of rabbit platelet rich plasma and resuspended in 5 ml of 50 mM Tris buffer pH 7.4. The suspension was subjected to thawing and freezing three times and centrifuged at 5000g for 15 min to remove cell debris⁹. 200 μ l of the supernatant (RPH) and 300 μ l of AA (300 μ M) were mixed and kept on ice. After 3 min an aliquot of 100 μ l was bioassayed. In one experiment L 8027 (1 μ M) was preincubated with RPH for 3 min at 22 °C before AA was added. The assay organs (rabbit mesenteric artery, rabbit aorta, rat colon and rat stomach) were calibrated with 15 ng and 7.5 ng of PGE_2 . Presence or absence of each component of the incubation mixture is marked with X or 0, respectively. L 8027 inhibited the generation of TXA_2 by RPH (contractile response of vascular strips were either inverted or suppressed) and had little effect on prostaglandin generation (contractions of rat colon remained substantially not changed).

8027 is a competitive, reversible inhibitor of TXA_2 synthetase and therefore an IC_{50} value is not an appropriate measure for its anti-enzymic activity. In our experiments with platelet microsomes we were not able to quantify the amount of PG endoperoxides in the incubation mixture and to calculate K_i value for L 8027. Inability of L 8027 to inhibit the ADP-induced platelet aggregation is in favour¹¹ of the proposed mechanism of anti-aggregatory action of L 8027.

In summary, in our experimental conditions L 8027 at 1 μ M significantly inhibits the generation of TXA_2 by platelet microsomes, by platelet homogenates and by platelet rich plasma. The same concentration of L 8027 does not influence the formation of PG-like material by BSVM, by RSVM and by platelet homogenates (Fig. 1) or the activity of cyclo-oxygenase in RSVM. In our preliminary experiments L 8027 (1 μ M) did not inhibit the spontaneous formation of prostacyclin (PGX, PGI_2) by the incubated rings of rabbit mesenteric arteries²⁷⁻²⁹. Therefore we conclude that L 8027 at concentrations of 1 μ M and lower is a selective inhibitor of TXA_2 generation in blood platelets.

Aspirin, indomethacin and other acidic cyclo-oxygenase

inhibitors are all being considered as anti-platelet drugs¹², though their pharmacological effects and side effects are of a broad spectrum. We suggest that non-acidic L 8027 which is a potent inhibitor of TXA_2 generation in platelets, can compete with cyclo-oxygenase inhibitors in the treatment of thromboembolic diseases.

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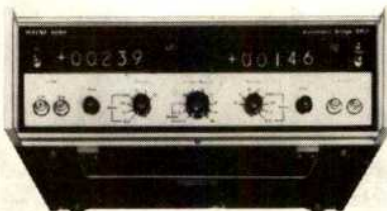
Effects of PGD_2 on canine renal function

In many tissues, especially the mammalian kidney, prostaglandin D_2 (PGD_2) is a significant product in the metabolism of the bisenoic prostaglandin endoperoxides^{1,2}. PGD_2 was originally reported to have negligible biological activity¹; but, subsequent workers noted that it produces vascular effects in several species^{3,4}. Although the effects in the kidney of the precursor, arachidonic acid, and other prostaglandins (PGs)⁵⁻⁷ have attracted much interest, the effects of PGD_2 have received little attention. Since PGD_2 may be an important product of arachidonate metabolism in the kidney^{8,9}, the renal response to an infusion of PGD_2 in the dog was examined and compared with the response to an infusion of PGE_2 . Whereas both PGs produced an increase in renal blood flow, only PGE_2 caused a diuresis and natriuresis. This suggests that PGD_2 in the kidney is primarily involved in the control of renal haemodynamics, and does not participate in the regulation of fluid and electrolyte excretion.

Table 1 shows the renal effects of the infusion of PGD_2 and PGE_2 . The infusion of either prostaglandin led to a pronounced increase in total renal blood flow (RBF). A marked diuresis and natriuresis accompanied the increase

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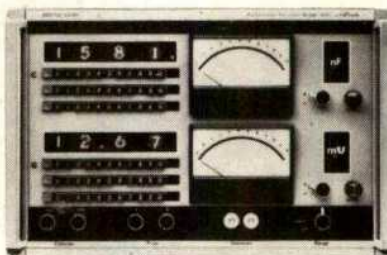
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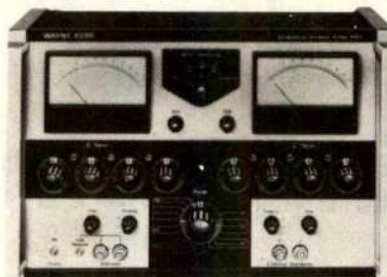
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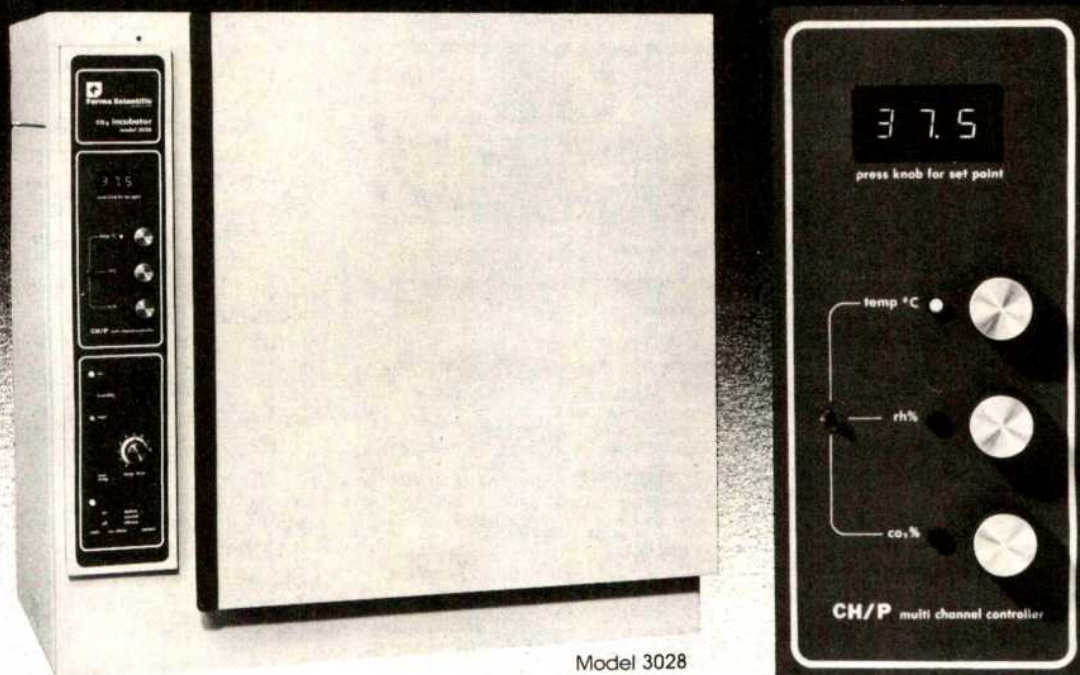


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in RBF produced by the infusion of PGE_2 , but not PGD_2 . The glomerular filtration rate (GFR) was not affected by the infusion of PGE_2 , but there was a significant reduction in this parameter during the infusion of PGD_2 . Both PGs significantly enhanced the levels of plasma renin activity in the renal vein (Table 1). The enhancement of RBF induced by the infusion of PGD_2 was caused by an elevation of blood flow to both the outer and inner cortical regions (Fig. 1). No significant alterations were observed in the contralateral kidney, the mean systemic blood pressure or the heart rate during the infusion of either prostaglandin.

Intrarenal infusion of PGD_2 and PGE_2 produced a similar degree of increase in total RBF. Although intrarenal blood flow during the infusion of PGE_2 was not reported in this study, Chang *et al.*⁷ observed that an intrarenal infusion of PGE_2 in the dog promoted an elevation of blood flow to the outer and inner cortical regions. In two experiments in which intrarenal blood flow was measured, the intrarenal infusion of PGE_2 produced alterations in intrarenal blood flow identical to those cited by Chang and co-workers⁷. The intrarenal infusion of PGD_2 also promoted an enhancement of outer and inner cortical blood flows confirming results obtained by Friesinger *et al.*¹⁰. These changes in intrarenal blood flow are similar to those produced by many vasodilators, but differ from the alterations produced by the infusion of the prostaglandin precursor, sodium arachidonate (unpublished observation of P.M.B. *et al.*). When sodium arachidonate is infused intrarenally there is an elevation of inner cortical blood flow, but no concomitant increase in the blood flow to the outer cortex.

As mentioned, the enhancement of RBF induced by the infusion of PGE_2 was accompanied by a marked diuresis and natriuresis, but infusion of PGD_2 had no effect on fluid and electrolyte excretion rates although it did induce changes in renal haemodynamics similar to those produced

by PGE_2 . Oelz *et al.*¹¹ have reported that intrarenal infusion of PGD_2 produced a natriuresis and diuresis in addition to an enhancement of RBF. (Since their data appeared in abstract form it is difficult at present to provide an explanation for the discrepancy between our work and theirs.) During PGD_2 infusion in the present study there was a reduction in GFR, which could have offset any direct tubular effects of PGD_2 . This is unlikely, however, since in subsequent studies we have found that a lower dose of PGD_2 ($0.04 \mu\text{g}$ per kg body weight per min) which produced effects qualitatively identical to those seen with the higher dose of PGD_2 (namely an increase in RBF without an accompanying diuresis or natriuresis) did not reduce the GFR. The absence of a natriuresis and diuresis during PGD_2 infusion could not have been due to a reduction in the renal perfusion pressure, since there was no change in the mean systemic blood pressure during the infusion of PGD_2 .

It is possible that PGE_2 promoted a diuresis and natriuresis because of a direct tubular inhibition of fluid and electrolyte transport. Several groups have observed that the renal prostaglandin system inhibits the action of vasopressin on the collecting duct^{12,13}, and in addition, in the isolated perfused rabbit cortical collecting tubule PGE_2 has recently been shown to inhibit directly the reabsorption of sodium¹⁴. The finding that PGD_2 did not alter the electrolyte and fluid excretion rates is somewhat unusual, since most renal vasodilators produce a natriuresis and diuresis which accompanies an increase in RBF^{15,16}. One exception to this is secretin, which was found to behave in a similar fashion to PGD_2 when infused into the renal artery¹⁷. It exhibited no effect on electrolyte and fluid excretion rates while increasing RBF, and when administered in low dosage there was no significant change in the GFR.

The infusion of both PGD_2 and PGE_2 led to an elevation

Table 1 Effects of PGD_2 and PGE_2 on canine renal function

Infusion periods	RBF ($\text{ml min}^{-1} \text{g}^{-1}$) (n = 5)	V ($\mu\text{l min}^{-1} \text{g}^{-1}$) (n = 8)	$\text{U}_{\text{Na}}\text{V}$ ($\mu\text{Eq min}^{-1} \text{g}^{-1}$) (n = 8)	FE_{Na} (n = 8)	$\text{C}_{\text{H}_2\text{O}}$ ($\mu\text{l min}^{-1} \text{g}^{-1}$) (n = 8)	GFR ($\text{ml min}^{-1} \text{g}^{-1}$) (n = 8)	RVPRA ($\text{ng ml}^{-1} \text{h}^{-1}$) (n = 6)
Control	3.2 ± 0.4	15 ± 5	0.9 ± 0.3	0.9 ± 0.3	-3 ± 4	0.65 ± 0.04	8.7 ± 3.9
PGD_2	$7.5 \pm 1.4^*$	15 ± 4	0.8 ± 0.2	1.0 ± 0.3	0 ± 4	$0.54 \pm 0.04^*$	$20.0 \pm 3.8^*$
Control	3.6 ± 0.4	16 ± 5	0.9 ± 0.3	1.0 ± 0.3	0 ± 3	0.58 ± 0.05	12.3 ± 4.1
	(n = 5)	(n = 7)	(n = 7)	(n = 7)	(n = 7)	(n = 7)	(n = 5)
Control	4.1 ± 0.7	16 ± 5	0.7 ± 0.3	0.7 ± 0.3	0 ± 4	0.60 ± 0.06	10.5 ± 4.0
PGE_2	$8.0 \pm 1.5^*$	$40 \pm 12^*$	$2.3 \pm 0.7^*$	$2.5 \pm 0.6^*$	$13 \pm 8^*$	0.68 ± 0.15	$21.2 \pm 7.1^*$
Control	3.6 ± 0.7	11 ± 2	0.9 ± 0.3	1.1 ± 0.3	-5 ± 2	0.58 ± 0.08	13.5 ± 5.9

Values for the right kidney are given as the mean $\pm$ the standard error. n, Number of animals; RBF, renal blood flow; V, urinary flow rate; $\text{U}_{\text{Na}}\text{V}$, urinary sodium excretion rate; $\text{C}_{\text{H}_2\text{O}}$, free water clearance rate; GFR, glomerular filtration rate; FE_{Na} , fractional excretion of sodium; RVPRA, renal vein plasma renin activity; * $P < 0.05$. Mean values for RBF and RVPRA were not obtained in all nine animals because of inaccurate flow readings in two and the inability to advance a catheter into the right renal vein for blood collections in two others. Mongrel dogs of either sex were fasted for 18 h before surgery and allowed free access to water. Anaesthesia was induced by intravenous pentobarbital (30 mg per kg body weight). An indwelling polyethylene catheter was inserted into the right femoral artery for monitoring systemic blood pressure and collection of blood samples. A catheter was introduced into the left femoral artery and advanced into the left ventricle for injection of radionuclide labelled microspheres ($15 \pm 5 \mu\text{m}$, New England Nuclear). A catheter was also placed in the left femoral vein and advanced into the right renal vein for collection of renal venous blood. A fourth catheter was introduced into the right femoral vein for the infusion of inulin used, to measure GFR. A suprapubic midline abdominal incision was made and both ureters were cannulated. A right retroperitoneal flank incision was made, and a hydraulic cuff and electromagnetic flowprobe were placed around the renal artery. Renal blood flow was monitored by a sine wave, non-occluding electromagnetic flowmeter (Biotronex Laboratories). The hydraulic cuff was used to establish and verify zero renal blood flow. A 25-gauge needle was inserted into the right renal artery proximal to the flowprobe and hydraulic cuff, and normal saline was infused at 0.5 ml min^{-1} . A period of 1 h was allowed for stabilisation before the commencement of the experiment. The study comprised five periods of 15 min duration each. Three 5-min urine collections were obtained during each period. Midway through each period, arterial samples for inulin, osmolality and sodium measurements and renal vein samples for renin determinations were collected. After the first 15-min control period, either PGD_2 ($0.4\text{--}0.8 \mu\text{g kg}^{-1} \text{min}^{-1}$) or PGE_2 ($0.4 \mu\text{g kg}^{-1} \text{min}^{-1}$), was infused into the renal artery for 15 min. Twenty minutes were allowed for renal function to return to control levels and then a second 15-min control period was begun. A 15-min infusion of either PGD_2 or PGE_2 (whichever had not been used before) immediately followed the second control period. The sequence of infusion of PGD_2 and PGE_2 was varied. A final 15-min control period was initiated 20 min after completion of the second PG period. Three bolus injections of differently labelled radionuclide microspheres (^{85}Sr , ^{141}Ce , ^{51}Cr) were administered immediately following the blood collections in the control period before infusion of PGD_2 , during the infusion of PGD_2 and in the control period immediately following PGD_2 infusion. At the end of the fifth period, the animals were killed and the kidneys were removed, weighed and sectioned according to the method of Slotkoff *et al.*²⁰. The sections were counted for radioactivity, to determine intrarenal blood flow. Renin levels were measured by radioimmunoassay. The level of significance was determined by the paired *t* test.

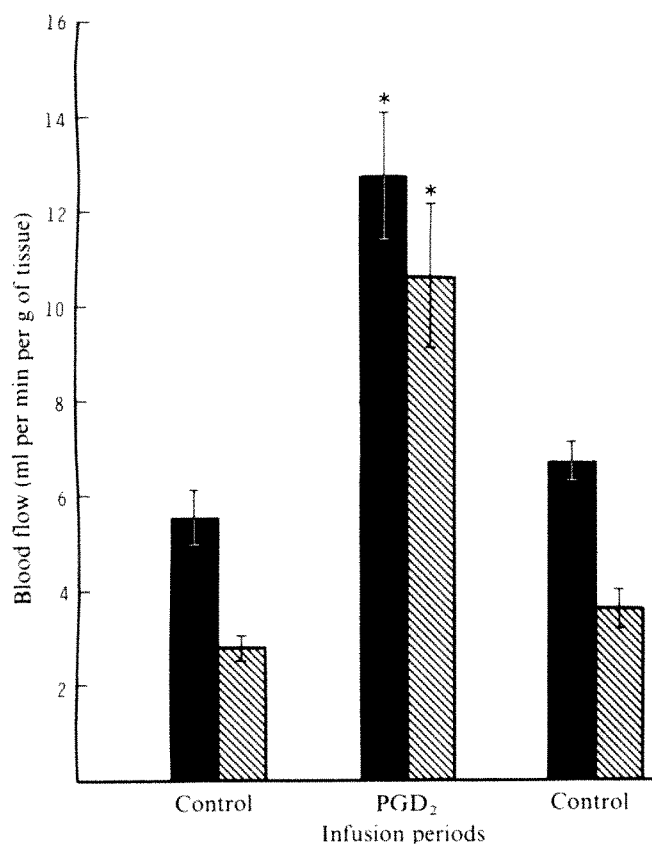


Fig. 1 The effect of PGD₂ on outer cortical and inner cortical blood flows (5 animals). Values for the right kidney are given as the mean $\pm$ the standard error. Outer cortical blood flow is represented by the solid bars and inner cortical blood flow is represented by the striped bars. * $P < 0.05$.

in renal venous plasma renin activity (PRA). It has been shown in an *in vitro* rabbit kidney preparation that arachidonate and several of the PGs directly stimulate renin release¹⁸. Arachidonic acid has also been shown to promote the *in vivo* release of renin in several species^{3,19}.

This study has shown that unlike PGE₂ and other vasodilators, an intrarenal infusion of PGD₂ alters renal haemodynamics without producing concomitant changes in electrolyte and fluid excretion. An infusion of PGD₂ also seems to stimulate renin release; whether this represents a direct action or is consequent to renal haemodynamics cannot yet be answered.

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Elevated serum levels of 1 α , 25-dihydroxycholecalciferol in lactating rats

LACTATION represents a challenge to regulation of calcium metabolism. Because of the high rate of calcium transfer to the milk, the lactating rat consuming a commercial stock diet maintains a reduced serum Ca level¹. The increased need for Ca seems to be met primarily by increased intestinal absorption of Ca (ref. 1). Since vitamin D is important for regulation of Ca metabolism, particularly during lactation², and since the circulating level of the active metabolite 1 α ,25-dihydroxyvitamin D₃ (1 α ,25-(OH)₂D₃) seems to be regulated according to Ca needs³, we determined by radioreceptor binding assay the serum levels of 1 α ,25-(OH)₂D₃ in vitamin D-fed lactating rats. We report here that these levels are two- to fourfold higher than those of non-lactating controls. Rats deprived of vitamin D during lactation fail to show elevated metabolite blood levels and develop marked hypocalcaemia, thus indicating the importance of the elevated blood level of 1 α ,25-(OH)₂D₃ for Ca metabolism during lactation.

1 α ,25-(OH)₂D₃ has been called the hormonal form of the vitamin⁴, because its formation in the kidney⁵ as well as its circulating level is apparently stringently controlled. Evidence for such regulation is the absence of an increase in the blood level of this metabolite in the face of a many-fold increase in the blood level of the precursor, 25-OHD₃, during chronic administration of excess vitamin D (refs 5, 6). Regulation of the blood level of 1 α ,25-(OH)₂D₃ may involve negative feedback of this metabolite on its synthesis in the kidney⁷. Factors that can stimulate renal 1 α -hydroxylation of 25-hydroxyvitamin D₃ (25-OHD₃) and cause an elevation of the 1 α ,25-(OH)₂D₃ level in the blood are hypophosphataemia^{3,8} and increased rate of secretion of parathyroid hormone^{3,8–10}, as well as hypocalcaemia independent of parathyroid hormone³.

Rats with litters of ten 6-d-old pups, and non-lactating females of the same age, were obtained from the Holtzman Company, where they had been maintained on a low vitamin D diet with 27% protein, 1.25–1.7% Ca and a Ca : P ratio of 1.5 : 1. On the seventh day of lactation, the adults were assigned randomly by weight to one of two dietary groups. One group received the basic diet consisting of 78% whole wheat flour, 14% casein, 4% corn oil, 2% Ca- and P-free salt mixture, 1.3% of vitamin D-free vitamin mixture (Nutritional Biochemicals) and 0.8% CaCO₃, with a final Ca and P content of 0.4% (–D diet). The other group received the basic diet to which had been added cholecalciferol (Sigma) at a concentration equal to that of Purina Laboratory Chow (5 IU per g of diet) (+D diet). Initially and at weekly intervals during the experiment, non-fasted adults were bled from the tail or by heart puncture and the adults and their litters were weighed. If the size of a litter fell below seven, the mother and pups were excluded from the experiment.

During a 4-d period (days 9–13) the food intake was measured and faeces were collected to determine the daily intake and rate of net absorption of Ca. Ca in food, faeces and serum was determined in a lanthanum chloride solu-

tion by atomic absorption spectrophotometry with a Perkin-Elmer 305 instrument. Phosphate (as inorganic phosphorus) was determined colorimetrically¹¹. Serum (3–4 ml per rat) was frozen for determination of $1\alpha,25-(\text{OH})_2\text{D}_3$ by a competitive radioreceptor binding assay which utilises the chick intestinal cytosol receptor and its association with chromatin. The $1\alpha,25-(\text{OH})_2\text{D}_3$ was first isolated from the serum as described by Hughes *et al.*³, and assayed according to recent modifications of the binding assay procedure¹². Triplicate assays were carried out on all samples, and the interassay variation was 10–15%. Studies with this assay show that vitamin D-deficient animals have undetectable levels of the hormone⁶ and measured circulating levels correlate with expected physiological modulations during dietary alterations of vitamin D (ref. 6), calcium and phosphate³.

Standard errors were calculated from analyses of variance of the data in each experiment and a one-tailed Student's *t* test was used to test significance of differences between mean values.

Table 1 contains the serum Ca levels on the seventh day, which marked the beginning of the dietary treatment, and the serum Ca, P and $1\alpha,25-(\text{OH})_2\text{D}_3$ levels on the 20th day. The seventh day values show that the lactating rats had significantly lower serum Ca levels than the non-lactating rats (experiments 2 and 3). Table 1 also shows that vitamin D-fed rats that had lactated for 20 d had elevated blood levels of $1\alpha,25-(\text{OH})_2\text{D}_3$. In experiment 2 the $1\alpha,25-(\text{OH})_2\text{D}_3$ blood levels of the lactating rats were over threefold higher than that of the non-lactating rats. The same lactating rats had significantly lower serum Ca and higher serum P levels than the non-lactating rats on the 20th day. When vitamin D was withheld from the diet for 2 weeks, lactating rats in experiments 1 and 2 had significantly lower serum $1\alpha,25-(\text{OH})_2\text{D}_3$ and Ca levels than vitamin D-fed lactating rats. This is in contrast to the vitamin D-deprived non-lactating rats (3) which showed no significant reduction in the serum levels of either $1\alpha,25-(\text{OH})_2\text{D}_3$ or Ca compared to their vitamin D-fed controls (experiment 3). Before the effect of vitamin D-deprivation (that is hypocalcaemia) was apparent in the rats in experiment 3 (days 9–13), intestinal absorption of Ca was 123 ± 2 mg per d or $62 \pm 4\%$ of Ca intake (mean $\pm$ s.e., $n=5$) for the lactating rats and 11 ± 2 mg per day or $15 \pm 3\%$ of Ca intake for the non-lactating rats. In another experiment (not shown) with rats that had been maintained on the +D diet and lactated for at least 3 weeks Ca absorption was comparable with that of the lactating rats in experiment 3, confirming the obser-

vation that calcium absorption is markedly elevated during lactation.

The present data represent the first demonstration of elevated serum levels of $1\alpha,25-(\text{OH})_2\text{D}_3$ during lactation. High levels of this hormone have also been found in young rats^{3,13} (as compared with adults) and in the laying hen in which the Ca needs also are increased¹². The serum $1\alpha,25-(\text{OH})_2\text{D}_3$ levels in the non-lactating rats in the present study are comparable with the levels reported for adult rats¹⁴.

The elevation of the $1\alpha,25-(\text{OH})_2\text{D}_3$ blood level in the vitamin D-fed lactating rats could have been due to the relative hypocalcaemia, which was clearly demonstrated, or to increased secretion of parathyroid hormone, which is a usual consequence of hypocalcaemia. Hypophosphataemia, on the other hand, can be ruled out as a stimulus in these rats since the serum phosphate values for the vitamin D-fed lactating rats were actually higher than those for the non-lactating controls. We should also consider the possibility that factors of importance for maintenance of milk secretion, such as thyroid and adrenal hormones and prolactin, also may contribute to elevation of the $1\alpha,25-(\text{OH})_2\text{D}_3$ blood level. In fact, prolactin injection in chicks has been shown to enhance both the activity of the renal 1α -hydroxylase¹⁵ and the circulating concentration of $1\alpha,25-(\text{OH})_2\text{D}_3$ (ref. 12).

While the present data are consistent with the idea that the elevated blood levels of $1\alpha,25-(\text{OH})_2\text{D}_3$ are essential for the maintenance of an increased rate of intestinal Ca absorption, it remains to be seen if a high rate of absorption also can be maintained in the vitamin D-deficient lactating rats perhaps by an action of parathyroid hormone^{16,17} or prolactin¹⁸ or other factors that may influence Ca absorption. Maintenance of serum Ca is apparently dependent on the elevated serum $1\alpha,25-(\text{OH})_2\text{D}_3$ level during lactation since severe hypocalcaemia ensued in vitamin D-deprived lactating rats that lacked the elevated $1\alpha,25-(\text{OH})_2\text{D}_3$ level. The level of $1\alpha,25-(\text{OH})_2\text{D}_3$ fell rapidly in the latter rats, if it can be assumed that this level was elevated during the first week of lactation before the vitamin D-deprivation. Since such deprivation did not affect this level in non-lactating rats, the present data are consistent with the idea that vitamin D metabolism is altered during lactation. In conclusion, it is suggested that elevation of the blood level of $1\alpha,25-(\text{OH})_2\text{D}_3$ takes place as a result of factors associated with the increased Ca demands of milk secretion, and that the elevated level of $1\alpha,25-(\text{OH})_2\text{D}_3$ is required for efficient regulation of Ca metabolism.

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Table 1 Serum calcium, phosphate and $1\alpha,25-(\text{OH})_2\text{D}_3$ levels in lactating and non-lactating rats

Experiment	Diet group	7th day of lactation		20th day of lactation		
		Ca (mg dl ⁻¹)	Ca (mg dl ⁻¹)	P (mg dl ⁻¹)	$1\alpha,25-(\text{OH})_2\text{D}_3$ (ng dl ⁻¹)	
1	Lactating	−D	9.8 ± 0.2	7.8 ± 0.4*	4.4 ± 0.3	5.8 ± 1.7
	Lactating	+D	9.6 ± 0.3	9.4 ± 0.5	5.9 ± 0.3	12.8 ± 1.2§
2	Lactating	−D	9.4 ± 0.1	7.6 ± 0.2*	3.7 ± 0.2	7.6 ± 0.6
	Lactating	+D	9.1 ± 0.1†	9.4 ± 0.2†	5.4 ± 0.2†	26.7 ± 0.6‡
3	Non-lactating	+D	9.8 ± 0.1	10.1 ± 0.2	4.6 ± 0.2	7.2 ± 0.6
	Lactating	−D	8.8 ± 0.1‡	6.9 ± 0.3*‡	3.8 ± 0.2	6.7 ± 0.7
	Non-lactating	−D	10.3 ± 0.1	10.1 ± 0.3	5.1 ± 0.2	4.6 ± 0.7
	Non-lactating	+D	10.5 ± 0.1	10.2 ± 0.3	4.7 ± 0.2	5.6 ± 0.9

The +D diet contained 5 IU D₃ per g diet and both +D and −D diets contained 0.4% Ca and 0.4% P. Non-lactating rats in experiment 2 were obtained as such from the supplier, while for experiment 3 rats were rendered non-lactating by removing their pups on the 8th day of lactation. The weight ranges for lactating and non-lactating rats were 240–390 g and 313–426 g, respectively. The mean weights for groups of rats within an experiment were not significantly different from each other ($P > 0.05$), neither were the weight gains by litters from −D and +D mothers. Each Ca and P value is the mean $\pm$ s.e. of 7–11 rats. Each $1\alpha,25-(\text{OH})_2\text{D}_3$ value is the mean $\pm$ s.e. of three or four pools of blood, each from three rats (except for lactating −D rats in experiment 1, two pools from five rats); determinations were in triplicate.

*Different from initial (as paired differences) and from lactating +D where appropriate, $P < 0.01$.

†Different from non-lactating, $P < 0.01$.

‡Different from non-lactating, $P < 0.001$.

§Different from lactating −D, $P < 0.025$.

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In vitro induction of binding sites for hCG in rat granulosa cells by FSH

THE granulosa cells (GC) of the Graafian follicle are considered to be the target of the cyclic surge of luteinising hormone (LH) which initiates ovulation and subsequent luteinisation of the follicle. It has been demonstrated that GC acquire receptors for LH during the final stages of follicular growth^{1,2}. In the immature hypophysectomised rat, treatment with oestrogen stimulates GC proliferation and growth of a large number of pre-antral follicles^{3,4}. Cells from these follicles are endowed with follicle-stimulating hormone (FSH) receptors⁵ and respond to FSH with increased progesterin production⁶. They contain few or no LH receptors, however, as reflected by their negligible ability to bind ¹²⁵I-hCG (ref. 7) or to respond to LH with increased progesterin production in culture (G. F. Erickson and A.N., unpublished). A dramatic increase of ¹²⁵I-hCG binding was demonstrated in GC following sequential treatment of immature hypophysectomised rats with oestradiol-17 β and FSH (refs 7, 8). To clarify the cellular mechanism of the induction of the LH receptors, we examined the induction of LH receptors by FSH *in vitro* and show that hCG binding sites can be induced by FSH in GC of pre-antral follicles in cultures of ovarian fragments, but not in isolated GC cultured as monolayers.

Wistar-derived immature hypophysectomised rats (28–30 d old) were each given four daily subcutaneous injections of 0.5 mg diethylstilboestrol in peanut oil (Hx-DES rats) and on day 5, the ovaries were excised aseptically. For organ culture, each ovary was sectioned into 8–12 fragments and these were placed on the grid of a Falcon organ culture dish and incubated⁹. For cell cultures, GC were isolated by puncture of follicles and gentle expression into the medium

and then grown as described by Nimrod and Lindner⁹. The following substances were added where indicated: hFSH (0.1 or 0.5 μ g ml⁻¹, LER-1801-3); androstenedione (0.1 μ g ml⁻¹); oestradiol-17 β (1.0 μ g ml⁻¹); insulin (5.0 μ g ml⁻¹, Squibb); and 8-bromo cyclic AMP (1.0 mM). When the culture period exceeded 48 h, the medium was changed with fresh addition of hormone. At the end of the culture period, the medium was removed and GC were collected using a rubber scraper (cell cultures) or collected from the ovarian segments as described above (organ cultures), and placed in buffered sucrose solution⁵. The cells were sedimented by centrifugation (500g for 8 min) and resuspended in a small volume of buffer by repeated passage through a Pasteur pipette. Highly purified hCG (13,600 U mg⁻¹, Serono, Italy) was labelled with ¹²⁵I (20–50 μ Ci per μ g hCG) by the lactoperoxidase method of Miyachi *et al.*¹⁰ and used in the assay of hCG binding⁵. Briefly, cell suspensions (50 μ l containing 2×10^5 to 5×10^5 cells) were incubated for 3 h at 37 °C in a shaking incubator together with 50 μ l of buffer containing 7–11 ng ml⁻¹ ¹²⁵I-hCG with or without an excess of unlabelled hCG (Pregnyl, Organon; 0.5 U per sample). The difference between radioactivity bound to the cells in the presence and absence of unlabelled hormone was taken to represent specific binding. Progesterone and 20 α -dihydroprogesterone accumulation in the culture medium was determined by radioimmunoassay using specific antibodies¹¹.

Specific binding of ¹²⁵I-hCG to the cultured cells (48 h) was very low (0.16 fmol per 10⁶ cells) even compared with that obtained with freshly collected cells (0.39 fmol per 10⁶ cells). Culture of these cells with FSH, or with FSH in combination with androstenedione, oestradiol-17 β or insulin, had no effect on ¹²⁵I-hCG binding, although the cells clearly responded to FSH with an increased release of progestins into the medium and androstenedione augmented this effect as previously reported⁶.

By contrast, exposure of ovarian fragments in organ culture to FSH (0.1–0.5 μ g ml⁻¹) for 48 h greatly augmented specific binding of ¹²⁵I-hCG to GC (1.19 $\pm$ 0.14 fmol per 10⁶ cells, mean $\pm$ s.e.m., $n=20$, compared with 0.26 $\pm$ 0.03, $n=22$ in untreated controls, $P<0.001$ by Student's t test; compare Table 1). Extension of the culture with FSH to 96 h resulted in a further increase in the number of ¹²⁵I-hCG binding sites (Fig. 1). Addition of androstenedione (Table 1)

Fig. 1 *In vitro* induction of hCG binding sites by FSH in GC during organ culture of ovaries from Hx-DES rats. Time course of increase in binding sites; $\blacktriangle$, FSH-treated (0.5 μ g ml⁻¹); $\bullet$, no hormone supplementation. Vertical bars, $\pm$ s.e.m. ($n=3$).

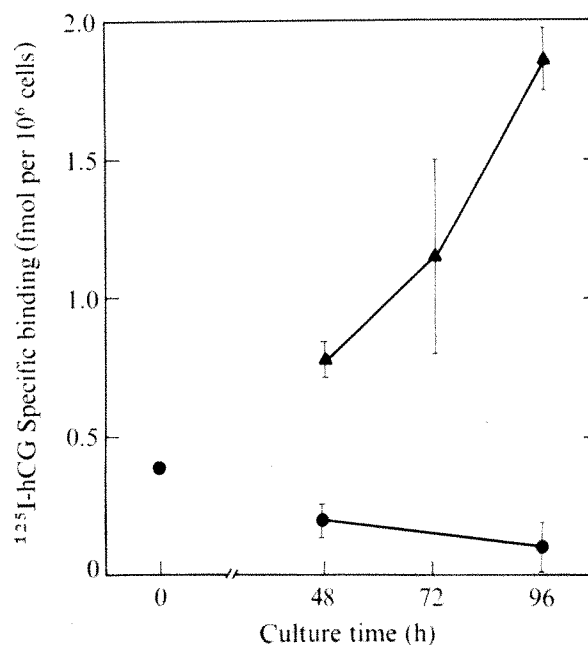


Table 1 The effect of FSH, androstenedione (Ad) and 8-bromo cyclic AMP on ^{125}I -hCG binding to the granulosa cells in organ cultures of ovaries from oestrogen-treated immature hypophysectomised rats.

Experiment no.	Culture conditions (48 h)	^{125}I -hCG binding (fmol per 10^6 cells)	n
1	No hormones	0.18 ± 0.03	5
	FSH ($0.1 \mu\text{g ml}^{-1}$)	1.23 ± 0.30	3
	FSH + Ad ($1 \mu\text{g ml}^{-1}$)	1.03; 1.47	2
2	No hormones	0.24 ± 0.05	5
	FSH ($0.1 \mu\text{g ml}^{-1}$)	1.17 ± 0.46	5
3	No hormones	0.45 ± 0.07	4
	FSH ($0.5 \mu\text{g ml}^{-1}$)	1.15 ± 0.27	4
4	No hormones	0.19 ± 0.04	4
	FSH ($0.5 \mu\text{g ml}^{-1}$)	1.12 ± 0.29	4
5	No hormones	0.26 ± 0.06	4
	FSH ($0.5 \mu\text{g ml}^{-1}$)	1.31 ± 0.23	4
	8-Bromo cyclic AMP (1.0 mM)	0.25 ± 0.08	4

Uptake by GC pooled from segments of two ovaries was determined in triplicate. Values shown are the mean $\pm$ s.e.m. of values obtained from n replicate cell pools or individual values where $n < 3$.

or oestradiol-17 β (data not shown) together with FSH did not modify the FSH effect. Culture of ovarian segments with 8-bromo cyclic AMP failed to induce hCG binding.

Organ cultures of ovarian fragments produced appreciable amounts of progestins (progesterone plus 20 α -dihydroprogesterone, 140 ± 26 ng per dish per 48 h; mean $\pm$ s.d.), but in contrast to isolated GC cultures, failed to respond consistently to FSH with increased progestin production, whether or not androstenedione or oestradiol-17 β were also added. 8-Bromo cyclic AMP likewise failed to stimulate progestin production in the organ cultures.

The present study represents the first demonstration of an *in vitro* induction of LH receptors in ovarian cells. The failure of 9-bromo cyclic AMP to mimic this FSH effect could signify either that this particular response is not mediated by the adenylate cyclase system, or that penetration of the follicle cells by the nucleotide was inadequate. The low level of ^{125}I -hCG binding in GC of Hx-DES rats offers an advantage over GC from mature follicles in that it eliminates the problem of loss of initial receptor sites during culture. Previously published data on stimulation of hCG binding in porcine GC cultures by FSH (ref. 12) could be interpreted as a reflection of better maintenance of pre-formed receptors, rather than the induction of new binding sites in the presence of this hormone. We were unable to induce the appearance of new receptor sites for hCG by isolated GC after culture with FSH, although the isolated cells responded to FSH with increased progestin production. The difference in the behaviour of GC when cultured alone or in intact follicles suggests the participation of other cellular components of the ovary, or their products, in the induction of the LH receptor by FSH.

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Morphological and biochemical heterogeneity of cholinergic synaptic vesicles

We present findings which seem to show that in an innervated perfused electric organ preparation from *Torpedo* the uptake of freshly synthesised acetylcholine by synaptic vesicles is linked to the discharge of previously stored transmitter by means of at least one cycle of exo- and endocytosis.

The electromotor synapse of *Torpedo* has provided a valuable model system for investigating the molecular basis of cholinergic transmitter storage and release¹. Although the numerous synaptic vesicles present in the nerve terminals seem to constitute a static and homogeneous population when the unstimulated tissue is viewed in the electron microscope, there is evidence that they are, in fact, morphologically and biochemically labile and heterogeneous. Thus, stimulation *in vivo* for short periods at 5 Hz caused marked vesicle depletion and reduction in size² and vesicles isolated from stimulated organs by submitting cytoplasmic extracts to density gradient centrifuging in a zonal rotor had less acetylcholine (ACh) and lower ACh/ATP ratios than those from unstimulated organs^{3,4}. On recovery from stimulation⁵, these changes are reversed, but vesicle numbers and diameters are more rapidly restored than vesicular ACh or ATP. The different types of vesicle do not differ sufficiently in physical properties to be sharply separated on a density gradient, though ACh/ATP ratios are observed to vary across the zonal vesicle peak⁴.

We aimed to modify the buoyant density of vesicles that had undergone exo- and endocytosis by incorporating into them dense material from the extracellular fluid. Sucrose, a constituent of a recently described *Torpedo* Ringer solution⁶, proved suitable for this purpose.

Innervated blocks of electric tissue 20–50 g in weight and comprising the territory of a single nerve were perfused with a physiological saline of osmolality made equal to that of elasmobranch tissue by the inclusion of sucrose (200 mM) and urea (400 mM) (ref. 6). The blocks were cannulated via the blood vessels which accompany the nerves and supply the same territory. Blocks were stimulated via the nerve either moist, in air, or submerged in the perfusion medium; no significant differences were observed with the two methods. The response of the organ was monitored by electrodes placed on its ventral and dorsal surfaces. Whole tissue samples were taken for electron microscopy and vesicles were isolated from cytoplasmic extracts of the blocks on zonal density gradients essentially as described before^{2,5,8}.

To avoid vesicle depletion due to repetitive stimulation we used a low stimulation frequency (0.1 Hz) at which the response of the tissue blocks decayed to about 20–30% of the initial response (27 V) after 1,800 stimuli and the total tissue ACh fell less, to about 30–40% of its initial value (of $1,300 \text{ nmol g}^{-1} \pm 367 \text{ s.e.m.}$, $n=3$). Vesicle numbers (per μm^2 of presynaptic terminal cross section) were not reduced; rather there was a small (up to 50%) increase which might have been caused by volume changes in the terminals during perfusion. There was, however, the same progressive appearance in whole tissue sections of a second population of vesicles about 25% smaller in diameter than the initial population, as occurred at 5 Hz.

When dextran (molecular weight 10,000, 6–10%) was added to the perfusion fluid, few or no dextran particles

were taken up into the vesicles during an initial 3 h perfusion period. When stimulation at 0.1 Hz was applied, however, there was a progressive uptake, mainly into the population of small vesicles (Fig. 1a). After 1,800 impulses, 80% of all vesicles (four experiments) were found to belong to the small diameter population and of these 91% contained dextran. Thus, transmitter release is accompanied by vesicle exo- and endocytosis.

To determine whether vesicles undergoing cycles of exo- and endocytosis were capable of taking up transmitter, ^3H -acetate (sodium salt; $0.5\text{--}1.5\ \mu\text{Ci ml}^{-1}$, $5\ \mu\text{M}$), a precursor of tissue ACh (ref. 7), was added to the perfusion medium; the vesicles were isolated⁸ and vesicular ACh was extracted for assay and separated from unesterified acetate for

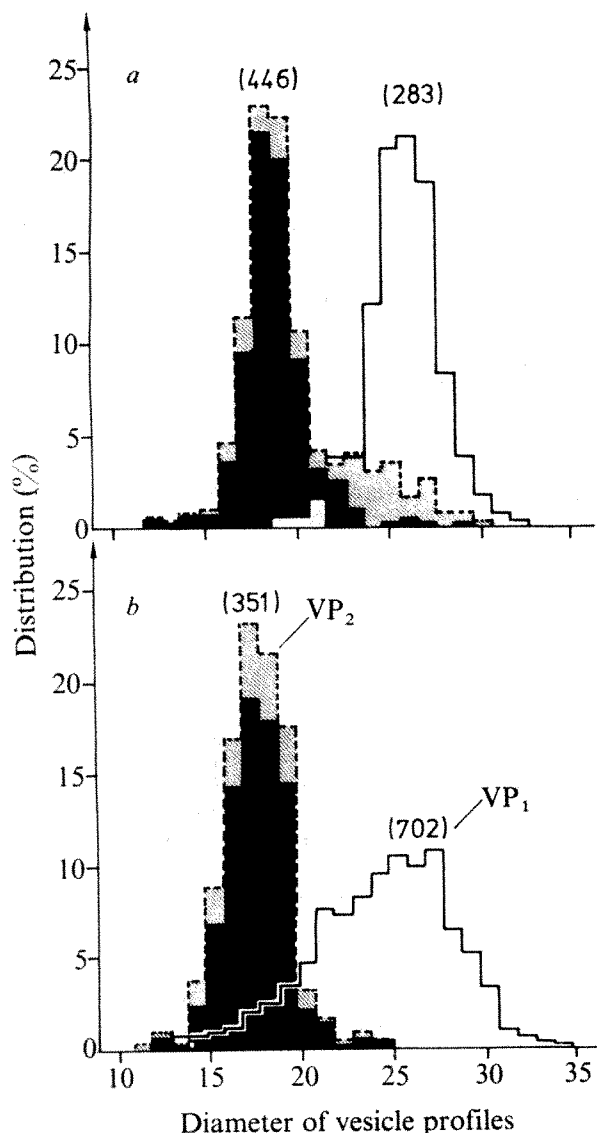


Fig. 1 *a*, Effect of stimulation (at 0.1 Hz) on synaptic vesicle profile diameters and the uptake of dextran into synaptic vesicles. The diagrams are normalised distributions of profile diameters in (continuous lines) unstimulated blocks and (dashed lines) blocks stimulated with 1800 pulses. Black blocks show the proportion of profiles of a given diameter that had a dextran particle in the lumen. Numbers in brackets give the number of vesicles measured. Only the small vesicles generated by stimulation contain dextran. *b*, Similar normalised distributions of vesicle profile diameters in vesicles isolated after 1,800 pulses. Note that fraction VP₁ (Fig. 2b) contains only the larger vesicle population whereas the smaller vesicles are recovered in VP₂. Electron-dense granules are restricted to the vesicles in VP₂ and the distribution of profile diameters in this vesicle fraction is similar to that of the newly formed small vesicle population shown in *a*. Abscissa: arbitrary scale.

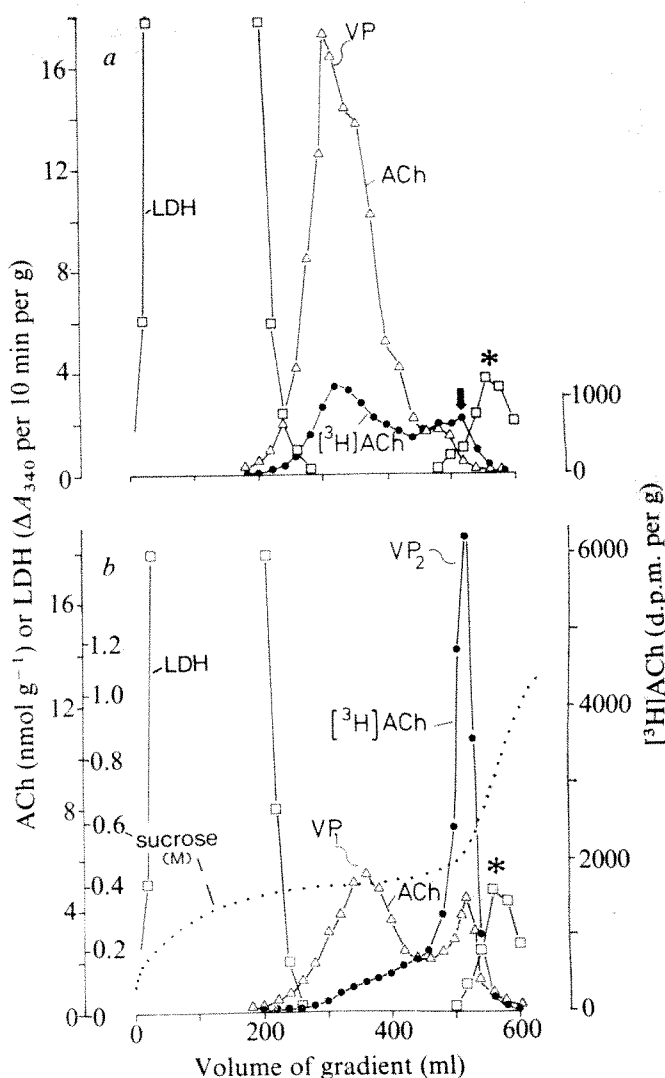


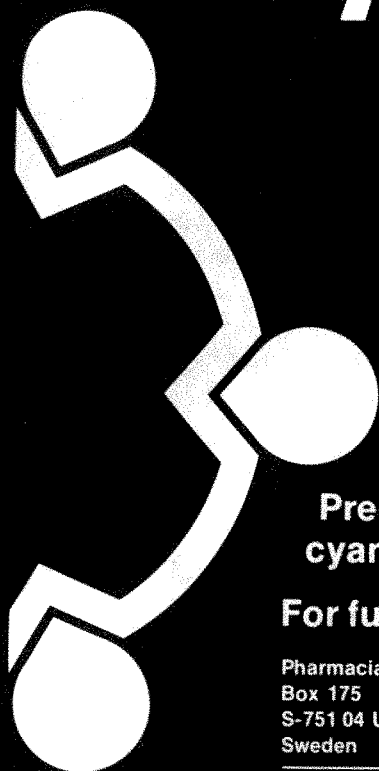
Fig. 2 *a*, Separation, in a zonal density gradient, of components of a cytoplasmic extract of an unstimulated tissue block showing peak (VP) of vesicular ACh, coincident peak and (arrow) shoulder of ³H-ACh. *b*, Result of stimulation, showing two peaks of vesicular ACh, VP₁ corresponding to, but smaller than VP in (a) and VP₂ corresponding to the shoulder in (a) at the inflexion of the sucrose gradient. Peak VP₂ is highly labelled and does not coincide with the peak of entrapped cytoplasm indicated by lactate dehydrogenase (LDH) (*).

counting by liquid ion-exchange partition⁹. Whereas there was some incorporation of radioactivity into the single peak of endogenous vesicular ACh derived from unstimulated blocks (VP, Fig. 2a), stimulation caused the appearance, in a denser region of the gradient, of a second highly radioactive peak of ACh (VP₂, Fig. 2b) containing about 20% of the total vesicular ACh coincident in position with a shoulder of radioactive ACh observed with the material from control blocks (arrow, Fig. 2a). Meanwhile the height of the peak (VP₁) corresponding to VP was reduced.

The specific radioactivity of the ACh of VP₂ was 16.6 ± 4.8 s.e.m. ($n=5$) times greater than that of VP₁ and 9.2 ± 2.4 ($n=4$) times than that of the parent cytoplasmic extract. Furthermore the ACh of the parent cytoplasmic extract had a specific radioactivity twice that of the tissue ACh (1.9 ± 0.1 , $n=7$). Similar results have been obtained with radioactive choline (J. Suszkiw and H.Z., unpublished) and the incorporation is blocked by hemicholinium-3.

Since the region of the gradient occupied by VP₂ overlaps the rising portion of a peak of membrane fragments, the possibility exists that VP₂ consists of cytoplasmic ACh sequestered within membrane bound particles. That this is

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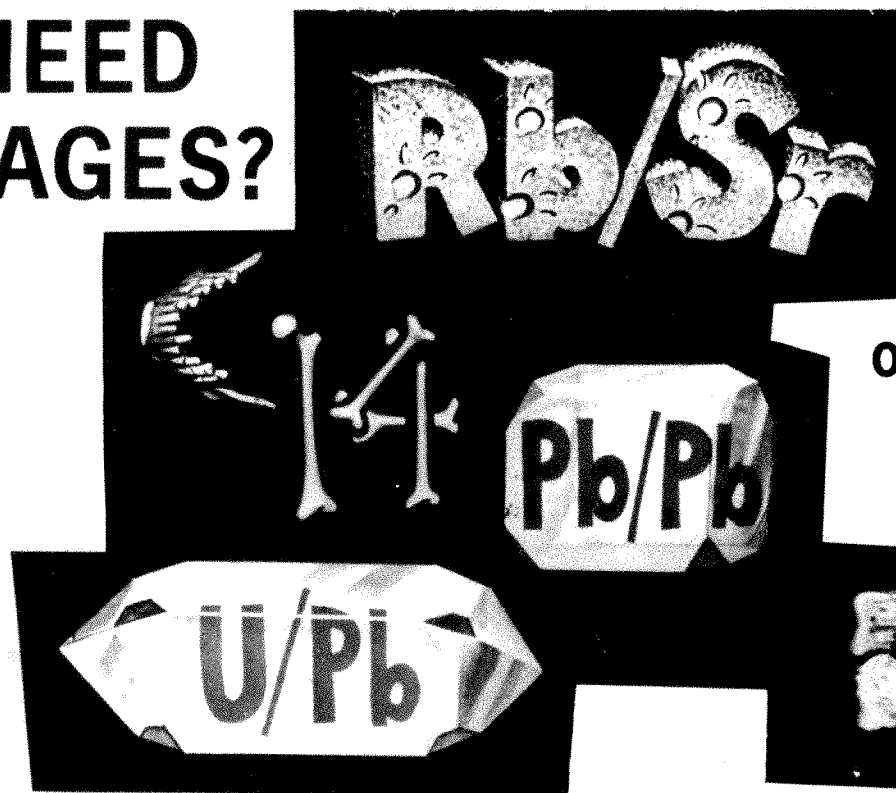
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not so was shown by its lack of coincidence with small peaks of the cytoplasmic markers lactate dehydrogenase (Fig. 2b) and choline acetyltransferase, and with a peak of separately prepared synaptosomes ('T-sacs')¹⁰ labelled with ¹⁴C-ACh and added to the cytoplasmic extract from stimulated tissue blocks (Fig. 3). There is good reason to assign the ACh of VP₂ to the population of small vesicles found mixed with membrane fragments in this region of the gradient. When dextran as well as ³H-acetate was perfused through stimulated tissue blocks, those vesicles could be readily distinguished electron microscopically from the larger membrane fragments by their content in electron-dense particles and Fig. 1b demonstrates that the diameter distribution of VP₁ and VP₂ is essentially the same as that between the large and small vesicle populations of intact terminals (Fig. 1a). Furthermore, electron-dense granules can be found only in the small vesicle population. The presence of dextran in the perfusion medium is not, however, essential for the separation of the two populations of vesicles; the essential component is sucrose.

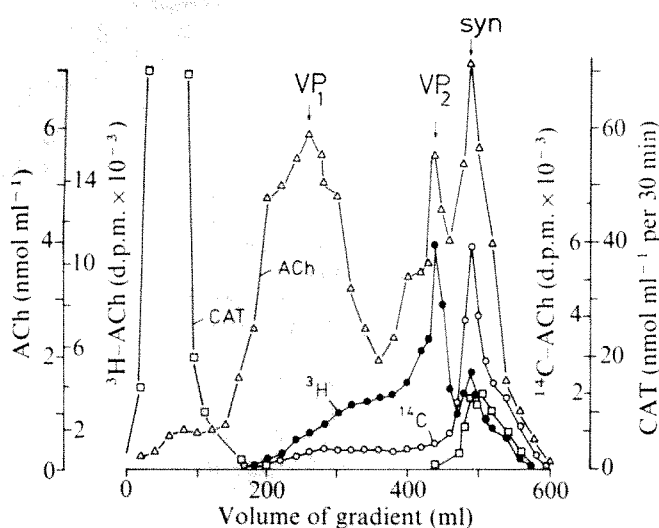


Fig. 3 Separation, in a zonal density gradient, of components of a cytoplasmic extract of a stimulated block to which a preparation of synaptosomes ('T-sacs') labelled with ¹⁴C-ACh, had been added. Note that VP₂ is well separated from the synaptosome peak (Syn) identified by its content of ¹⁴C-ACh and choline acetyltransferase (CAT).

These results seem to show (1) that *Torpedo* electromotor synaptic vesicles, like brain cortex vesicles¹¹, are metabolically heterogeneous with respect to ACh uptake. (2) That the subpopulation of vesicles taking up newly synthesised ACh has undergone at least one cycle of exo/endocytosis. And (3), that such vesicles can be separated from the main population by an artificially induced alteration of density, a technique similar to that used¹² to separate liver lysosomes from mitochondria. The implications of these findings for the vesicle theory of transmitter storage and release¹³ are now being examined.

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Interference by bromodeoxyuridine with differentiation in a prokaryote

ENDOSPORE formation in bacteria can be regarded as a primitive form of cellular differentiation as the mature spore has many features, both structural and biochemical, which distinguish it clearly from the growing cell. The extent to which bacterial sporulation can be used as a simple model for development in higher organisms depends on the similarity or otherwise of the control processes which influence cellular differentiation in both cases. It would thus seem important to draw as close an analogy as possible between prokaryotic and eukaryotic differentiating systems. With this in mind, the effect of 5-bromo-2'-deoxyuridine (BUdR) on growth and sporulation in *Bacillus subtilis* has been investigated. When incorporated into the DNA of eukaryotic cells, BUdR (an analogue of thymidine, TdR) selectively affects processes associated with differentiated and embryogenesis, without significantly altering cell growth or gross RNA and protein synthesis^{1–3}. The effects seem best explained by an alteration in transcription^{2,4,5}, possibly caused by tighter binding of chromosomal proteins^{6–8}. This may also be true for prokaryotes, because purified *lac* repressor and catabolite gene activator protein from *E. coli* bind more tightly to BUdR-substituted DNA⁹. Sporulation in *B. subtilis* is controlled to a considerable extent by sequential activation of genes, many of which remain repressed during vegetative growth (for review see ref. 9). We report here that BUdR, at a concentration which leaves growth unaffected, markedly reduces the capacity of *B. subtilis* to sporulate. This effect is comparable with the known interference of this analogue with cellular differentiation in eukaryotes.

B. subtilis 168 (*thy*[−]*A*, *trpC2*) was used as the parent strain, but even partial replacement of the necessary TdR in the medium by BUdR inhibited growth. To overcome this problem a mutant was isolated which still retained the original *thy*[−]*A* mutation, but was much less susceptible to the toxic effects of BUdR. This BUdR-tolerant strain (BUt-32) was able to grow normally (doubling time 36 min) in a rich casein hydrolysate (CH) medium¹⁰ with a BUdR:TdR ratio of 7.5 μg ml^{−1}:1.0 μg ml^{−1}. At higher ratios the growth rate was progressively affected. BUt-32 was selected by a procedure similar to that described for the isolation of 5-bromouracil (BU)-tolerant mutants of *B. subtilis*¹¹. Like the BU-tolerant strains, BUt-32 was unable to grow for more than a few generations with BUdR alone, and a small amount of the natural nucleoside had to be present during growth and sporulation. The basis of the increased resistance to analogue toxicity has not been investigated in either case.

The buoyant density in CsCl of DNA prepared from BUt-32 cells grown with TdR alone was compared with that from cells grown with BUdR and TdR (7.5:1.0). The degree of BUdR substitution can be estimated from the difference in buoyant density between poly d(A-T) and poly d(A-BU) which is 200 mg ml^{−1} (ref. 12). Because *B. subtilis* 168 DNA contains only 28% TdR residues¹³ compared with 50% in poly d(A-T), complete BUdR substitution should give a density increase of about 112 mg ml^{−1}. The density difference found averaged 35 mg ml^{−1} and in separate experiments varied between 40 and 28 mg ml^{−1}. This represents an average 30% substitution by BUdR, a value compatible with earlier results which showed a marked preferential

Table 1 Occurrence of sporulation events in cells supplemented with TdR plus BUdR or TdR alone

Growth and resuspension of cells in media containing*	Exoprotease† (U ml ⁻¹ of culture)	Alkaline phosphatase† (U ml ⁻¹ of culture)	Viable count‡ (colonies per ml × 10 ⁻⁵)	Heat resistance‡ (colonies per ml × 10 ⁻⁵)
TdR (8.5 µg ml ⁻¹)	0.08	0.21	4,030	818
TdR (1 µg ml ⁻¹) + BUdR (7.5 µg ml ⁻¹)	0.14	0.25	3,590	9

*Cells of BUt-32 growing exponentially in rich CH medium were resuspended in a poor minimal salts medium to initiate sporulation¹⁰. Samples of the cultures were assayed at hourly intervals for exoprotease activity¹⁵ and alkaline phosphatase activity¹⁶. Viable count was determined by dilution and plating on nutrient agar. Heat-resistance was determined in the same way, after heating a cell sample at 85 °C for 15 min. The values shown are the results of a typical experiment.

†Maximum values obtained, 4 h and 6 h after resuspension for exoprotease and alkaline phosphatase respectively. 1 unit of enzyme activity is defined as a change in absorption of 0.1 min⁻¹ at 595 nm and 410 nm for exoprotease and alkaline phosphatase respectively.

‡Measured 8 h after resuspension of cells.

uptake and incorporation by *B. subtilis* of the natural nucleoside, TdR, over BUdR^{11,14}.

The effect of this degree of substitution on the ability of the cells to sporulate was investigated (Table 1). During sporulation two early biochemical marker events, exoprotease (Pr) activity and alkaline phosphatase (AP) activity, were assayed and heat-resistance was used as a measure of the number of mature spores formed. The presence of BUdR had the effect of markedly increasing the activities of both Pr and AP over the control values, but the final yield of mature spores was reduced to about 1% of that of the control culture. The low degree of sporulation was not due to loss of viability by the cells incorporating BUdR (Table 1) or to delayed sporulation, for prolonged incubation (24 h) failed to increase the percentage spore yield relative to the control. Prevention of DNA replication is known to prevent sporulation^{17,18}, but DNA synthesis, measured by uptake of ¹⁴C-TdR into acid-insoluble material, continued in a similar manner in both cultures for 2 h after initiation to sporulation (results not shown).

Examination of cells by electron microscopy 7 h after initiation, when the bulk of the control cells had formed readily identifiable spores, showed that BUdR incorporation prevented most cells from reaching the stage of spore septum formation (stage II). Most of the cells incorporating BUdR were normal in length and had nuclear material in the form of an axial filament. Rarely, cells were seen with wall protrusions or 'spikes' at one pole (a recognised stage preceding spore septum formation¹⁹), but generally the cells had no characteristics of sporulating cells. The occasional elongated cell or multiseptate cell was also observed.

This apparent interference by BUdR with an early stage of sporulation was confirmed by exposure of cells grown with TdR alone to BUdR at intervals after initiation to sporulation (Table 2). Cells initiated in the presence of the analogue had a markedly reduced capacity to form spores, only slightly better than that exhibited by cells grown as well as sporulated with BUdR. Initiation of cells in resuspension medium with TdR alone followed by transfer to resuspension medium containing BUdR 1 h after initiation gave a much increased spore yield. By 2 h after initiation in TdR medium exposure of cells to BUdR had essentially no effect on subsequent sporulation. Conversely, cells grown with BUdR, but initiated to sporulation in its absence exhibited a much increased capacity to sporulate, roughly about half that of control cells previously grown with TdR alone (Table 2). This indicated that BUdR incorporation during growth had little subsequent effect on sporulation if initiation occurred in the absence of the analogue. Initiation in the presence of BUdR followed by transfer of cells to a medium containing TdR alone 1, 2 or 3 h later produced a much reduced spore yield when assayed at 8 h, but by 24 h the level of sporulation had recovered almost to that found in control cells. This recovery indicated that the inhibitory effect of BUdR on

sporulation was reversible on removal of the analogue followed by continued incubation in the presence of TdR alone.

Sporulation was induced in these experiments in shift-down conditions involving transfer of cells from a rich to a poor medium. This would have necessitated the general derepression of many genes involved in biosynthetic processes. If BUdR incorporation was affecting transcription generally, the effect on sporulation might be caused, for example, by lack of transcription for a required amino acid rather than an effect on sporulation itself. This explanation seems unlikely for two reasons. First, BUt-32 grew normally (doubling time 65 min) in the minimal resuspension medium supplemented with glucose (0.2%) with a BUdR: TdR ratio of 7.5 µg ml⁻¹: 1.0 µg ml⁻¹. After 24 h only the occasional spore was detected in this medium, yet more than 50% of the cells in a culture grown in the same medium with TdR alone sporulated. Second, when L-histidine (500 µg ml⁻¹) was included in the normal resuspension medium in an experiment similar to that described in Table 1, the rate of synthesis of the inducible enzyme histidase was the same whether the cells had incorporated BUdR or not.

The precise nature of the sporulation-associated events affected by BUdR incorporation is unknown, but certain

Table 2 Effect on spore yield of removal or addition of BUdR at intervals after initiation of cells to sporulation

Time of removal of cell sample after resuspension* (h)	Spore yield†			
	Removal from TdR to TdR + BUdR medium		Removal from TdR + BUdR to TdR medium	
	8 h	24 h	8 h	24 h
0	37	87	280	390
1	310	630	24	420
2	580	1020	13	380
3	710	1040	16	420

*Parallel cultures (50 ml) of BUt-32 were grown in CH medium in the presence of either TdR (1 µg ml⁻¹) + BUdR (7.5 µg ml⁻¹) or TdR (8.5 µg ml⁻¹). To initiate sporulation 10-ml and 40-ml portions of each culture were centrifuged and resuspended in minimal salts medium. For cells grown in the presence of TdR alone the 40-ml portion was resuspended with TdR (8.5 µg ml⁻¹) and the 10-ml portion with TdR (1 µg ml⁻¹) + BUdR (7.5 µg ml⁻¹); the latter represented a time zero transfer to the new medium. Cells grown with TdR + BUdR were treated in a similar manner except that the 40 ml portion was resuspended with TdR + BUdR and the 10 ml portion with TdR only. At hourly intervals for the next 3 h further 10-ml samples were removed from each 40 ml culture, the cells collected on a membrane filter and then resuspended in 10 ml pre-warmed salts medium such that cells previously exposed to TdR alone were introduced into TdR + BUdR medium and vice versa.

†Heat-resistant colonies per ml × 10⁻⁵ determined 8 h and 24 h after resuspension as described in the legend to Table 1. Values for control cultures were 620 (8 h) and 1,130 (24 h) for cells grown and sporulated in the presence of TdR alone and 14 (8 h) and 41 (24 h) for cells grown and sporulated in the presence of TdR + BUdR.

observations deserve comment. The increase in AP activity during sporulation is normally associated with stage II (ref. 21), but BUdR incorporation allowed the increase in activity without the accompanying morphological changes. In addition, both Pr and AP activities were markedly increased over control values. This overproduction of enzyme activity is reminiscent of that seen in certain *spo* mutants⁹ and has also been observed during BUdR incorporation in eukaryotes (noted in refs 7 and 8). The inhibitory effect of BUdR on sporulation only occurred within an initial 2 h after initiation, when DNA replication was in progress, and the effect was reversible on removal of the analogue. Sensitivity to BUdR during a restricted interval of development and reversibility of its effects by further rounds of DNA replication in its absence have been observed in many eukaryotic systems^{1,2}. The presence of BUdR during the growth phase of the cells before the induction of sporulation had only a marginal effect on their subsequent sporulation capacity. This raised the possibility that BUdR inhibition of sporulation was primarily a secondary effect not related to its incorporation into DNA. For example, effects on the cytoplasmic membrane might be expected to interfere more with sporulation than with growth²⁰. BUdR had no effect on the sporulation capacity of wild-type *B. subtilis*, however, which suggests that a simple phenotypic effect of this sort is unlikely. At present it must be assumed that the inhibitory effect of BUdR is a consequence, for the most part, of its incorporation into DNA during chromosome replication at the onset of sporulation. This incorporation might interfere with the regulation of the process in some way, perhaps by causing an alteration in transcription due to tighter binding of regulatory proteins or by an alteration in the stability of chromosome-membrane complexes as has been suggested for other systems^{1,2,20}.

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Transcription of a mammalian sequence under phage λ control

RECOMBINANT DNA techniques have recently enabled bacterial plasmids carrying eukaryotic sequences synthesised *in vitro* from purified mRNAs to be constructed and cloned in *Escherichia coli*^{1–10}. We show here that a rabbit globin sequence is not transcribed in *E. coli* when carried by the pCR1 plasmid. However, after transfer into phage λ , the globin sequence is transcribed under λ control. Thus, there is no basic hindrance to the transcription of a specific mammalian sequence in *E. coli*.

Recombinant plasmids containing globin cDNA obtained as previously reported by us¹, carried only

a relatively short part of the globin sequence. For the construction of another series of plasmids¹, much longer double-stranded globin DNA molecules were synthesised from cDNA copies of purified globin mRNA with reverse transcriptase in a 'self-priming' reaction⁸. A similar procedure for the synthesis of double-stranded DNA from cDNA has been independently devised by others^{6–9}. Plasmids were thus obtained with the α or the β globin gene from rabbit⁷ and mouse¹⁰.

The protocol used for these experiments^{1,7,10} involved the reconstruction of an *EcoRI* site on each side of the integrated sequence. Analysis of many hybrid plasmids revealed that these two *EcoRI* sites were present only rarely, but were present, however, on one of the rabbit plasmids, pCR1 β G19, which carries the entire β globin sequence. Since there is also an *EcoRI* site within this sequence, digestion of pCR1 β G19 DNA yields, in addition to the parent plasmid DNA, two smaller fragments, A and B, 410 and 210 base pairs long respectively, which correspond to the 5' (A) and 3' (B) ends of globin mRNA¹.

To determine whether the globin sequence is transcribed in *E. coli* harbouring pCR1 β G19, unlabelled RNA was extracted from exponentially growing cells, hybridised with ³H-globin cDNA, and the hybrids monitored with nuclease S1. There was little or no detectable globin RNA in conditions which we estimate would allow the detection of less than one molecule of globin RNA per bacterial cell (Table 3, line 1). We then explored the possibility that the rabbit sequence could be transcribed in *E. coli* when carried by another vehicle such as phage λ .

pCR1 β G19 DNA was digested to completion with *EcoRI*, and the fragments were recombined with the DNA of

Table 1 Characterisation of lysogens made from recombinant phages

DNA extracted from	³ H-globin DNA hybridised (c.p.m.)	Hybridisation ratio long—short/short	Recombinant structure
C600	8	—	—
C600 (pCR1 β G19)	663	0.17	pCR1-A-B
C600 (λ 1)	564	0.17	λ B
C600 (λ 2)	601	0.53	λ A
C600 (λ 3)	578	0.47	λ A
C600 (λ 4)	601	0.50	λ A
C600 (λ 5)	525	0.11	λ B
C600 (λ 6)	547	0.19	λ B
C600 (λ 7)	636	0.58	λ A
C600 (λ 8)	558	0.11	λ B
C600 (λ 11)	557	0.20	λ B
C600 (λ 12)	552	0.15	λ B
C600 (λ 13)	562	0.15	λ B
C600 (λ 14)	11	—	—
C600 (λ 15)	637	0.09	λ B
C600 (λ 16)	623	0.49	λ A
NoDNA	9	—	—

pCR1 β G19 DNA was purified and digested by *EcoRI* as described¹. It was next recombined, in a 3:1 molar ratio with *EcoRI* digested DNA of phage 589, in the presence of T4 DNA ligase at 4 °C (refs 11, 18). The calcium method (slightly modified, as described in refs 11 and 18) was used for transfection in C600_r⁺ m_k⁺. Infective centres were plated in the presence of W3110 *polA*[−] in a 1:1 ratio with C600_r⁺ m_k⁺. On the mixed lawn, *red*[−] phages form small turbid plaques at 37 °C, which are easily scored by stabbing on to C600 and W3110 *polA*[−] (ref. 11). 14 *red*[−] phages (the putative recombinants) were re-isolated twice; stocks were made, and lysogens were isolated from a lawn of C600 spotted at 32 °C with about 10⁸ phages. After purification, the lysogens were grown at 32 °C in broth. The DNA of the 14 independent lysogens was then extracted from 5 ml of culture, immobilised on nitrocellulose filters and hybridised with 1,000 c.p.m. of ³H-globin cDNA (7 × 10⁶ c.p.m. μ g^{−1}, prepared as in ref. 8), for 18 h at 37 °C in 2 × SSC–50% formamide pH 7.2; the reaction volume was 4 μ l (refs 1, 14, 15). Note that the ³H-cDNA, made from a mixture of α and β globin RNAs, consists of about 2/3 of β chain DNA. The table also shows the ratio (long—short)/short of c.p.m. hybridised with long or short cDNA fractionated in a sucrose gradient as described in the text.

Table 2 Transcription of the globin sequence assayed by filter hybridisation

³ H RNA extracted from	Input (c.p.m.)	c.p.m. hybridised to				Globin-specific c.p.m.	Globin/ λ
		λ DNA	pCR1 β G19	pCR1	no DNA		
W3350 (λ CI857 <i>cro</i> ⁻ <i>P</i> ⁻ <i>int</i> ⁻)	42 °C	228,000	50,440	150	185	120	—
C600 (λ 2)	32 °C	475,000	180	80	65	20	—
	42 °C	610,000	5,840	280	90	30	190
C600 (λ 3)	32 °C	945,000	160	95	110	30	—
	42 °C	382,000	4,765	400	125	35	275
C600 (λ 4)	32 °C	1,400,000	210	80	135	40	—
	42 °C	431,000	3,810	170	70	20	100
C600 (λ 7)	32 °C	300,000	190	110	110	40	—
	42 °C	196,000	4,000	165	80	30	85

Four lysogens were grown in 63 medium supplemented with glucose, casaminoacids and vitamin B1 (ref. 16), for pulse labelling of 10 ml of culture with ³H-uridine (CEA, 25 Ci mM⁻¹, 20 μ Ci ml⁻¹). The RNAs were extracted as before¹⁶ and hybridised at 37 °C for 4 h, in a final volume of 4 μ l of 2 $\times$ SSC–50% formamide, to filters carrying 1 μ g of λ , pCR1 β G19 or pCR1 DNA, or no DNA. Filters were processed as usual¹⁵. There is no cross hybridisation between the phage, plasmid and globin sequences as shown in the first line of the table, where λ RNA from a overproducing strain¹⁸ was labelled and hybridised in similar conditions.

phage 589 in our collection. This phage was derived from λ plac5 by a series of *in vivo* and *in vitro* manipulations (P.K. and D.G., in preparation). It has lost the *EcoRI* *lac* fragment (a 14% deletion) and has only one *EcoRI* sensitive site located within the *red* gene of λ (ref. 12). Integration of foreign DNA at this site makes the phage *red*⁻ so that recombinants are easily scored as small plaque formers on a mixed lawn (1:1 of *polA*⁺ and *polA*⁻ bacteria (ref. 12 and P.K. and D.G. in preparation). Recombinants of phage 589 (which carries the *cl*857 mutation) lysogenise easily at 32 °C, at least at high multiplicity, in spite of the partial cold-sensitive character of such phages (F. Bregegere, J. Leal and P.K., in preparation). Since pCR1 DNA is too large to be accommodated within phage 589, the only recombinants which could be obtained are those carrying the A or B globin fragment, or both.

Recombinants were isolated, and a lysogen made with ³H-globin cDNA (ref. 1). Of 14 strains examined, 13 carried the lysogens, denatured, and immobilised on nitrocellulose

filters for hybridisation in formamide¹³ under paraffin oil¹⁴ with ³H-globin cDNA¹. Of 14 strains examined, 13 carried a globin sequence. Hybridisation was further carried out with cDNA fractionated in a sucrose gradient into short cDNA (< 250 nucleotides) or long cDNA (> 250 nucleotides) and with ³H-pCR1 β G1 DNA. This allowed the identification of phages carrying the A or the B fragment, or both. In five strains out of thirteen, hybridisation with short cDNA was significantly reduced. These strains, therefore, are of the λ -A type, while the others are λ -B, or λ -A-B. Monitoring with ³H-pCR1 β G1 DNA, a plasmid which carries only a short part of the globin sequence¹, indicated preferential hybridisation with DNA from the λ -A strains. This suggests that the portion of the globin sequence in pCR1 β G1 is mostly in the A region, and that (with two possible exceptions) the eight other strains are λ -B rather than λ -A-B.

Is the globin sequence transcribed in non-induced or induced lysogens, and if so, is it transcribed from the correct or the anti-sense strand?

Attention was first focused on four λ -A strains. We took advantage of the availability of the globin sequence in two unrelated molecular vehicles (pCR1 and λ) (which allow it to be produced in large amounts) to assay transcription from λ -A by hybridisation to a large excess of pCR1 β G19 DNA. Four λ -A lysogens were pulse labelled with ³H-uridine at 32 °C (3 min) or at 42 °C (2 min after an incubation of 3 min at 42 °C; early transcription in the *red* region is known to be maximal at about this time¹⁵). The radioactive RNAs were extracted and hybridised to excess pCR1 β G19 and pCR1 DNA on filters. In all cases transcription of the globin sequence occurred in the induced, but not in the non-induced lysogens (Table 2). The rough assumption can be made that about 70% of the radioactivity hybridised to λ DNA reflects the *l* strand transcription of about 16% of the λ chromosome, that is, 7,000 base pairs. The transcription of 410 base pairs of globin sequence should correspond to about 3.5% that of λ , in relatively good agreement with figures of 2 to 5% derived from data in Table 2.

To determine whether the coding strand is transcribed in at least some of the strains, unlabelled RNA was extracted from non-induced lysogens and lysogens induced for 7 min. The unlabelled RNAs were hybridised with ³H-cDNA. On treatment with nuclease S1, protection of the ³H-cDNA is observed in two strains out of four, demonstrating that, in these two strains, the coding strand is transcribed (Table 2). When similar experiments were carried out with the other strains, transcription of the coding strand was observed in a total of eight strains out of the thirteen examined.

On methodological grounds, the above results illustrate the use of two unrelated vectors (λ and pCR1) carrying a

Table 3 Transcription of the globin sequence assayed by hybridisation to ³H- β globin cDNA.

RNA extracted from C600 harbouring	+S1	-S1	% resistance
pCR1 β G19	63	296	12
λ 1 I	172	341	45
λ 2 I	73	318	14
λ 3 { NI	26	332	0
I	30	346	0
λ 4 { NI	27	357	0
I	198	335	55
λ 5 I	48	305	3
λ 6 { NI	49	274	3
I	169	296	53
λ 7 { NI	24	311	0
I	208	344	60
λ 8 I	43	277	1
λ 11 I	153	296	45
λ 12 I	54	255	5
λ 13 I	174	275	57
λ 14 I	49	273	3
λ 15 I	219	262	81
λ 16 I	220	330	63

200 ml of C600 *r*_k⁻ *m*_k⁻ *recBC*⁻ (pCR1 β G19) were grown (in broth at 37 °C) as well as 200 ml of the various lysogens (grown in broth at 32 °C and induced at 42 °C for 7 min) and in some cases, 200 ml of the non-induced lysogens. Cells were collected at a density of 4 $\times$ 10⁸ bacteria per ml. The RNAs were extracted as before^{16,18} and concentrated to about 1 mg ml⁻¹. 100 μ l were hybridised for 16 h at 70 °C with 700 c.p.m. of ³H-globin cDNA copied from the β chain (7 $\times$ 10⁶ c.p.m. μ g⁻¹ as before) in a final volume of 120 μ l in 2 $\times$ SSC. After incubation, the samples were divided into two, and precipitated with TCA after previous or no treatment with nuclease S1. A background of 30 c.p.m. was subtracted before calculating the percentage resistance.

I, induced; NI, non-induced.

similar DNA piece as a general tool for transcriptional studies (DNA from one recombinant can serve to monitor transcription from the other). Our results also show that *Eco*RI sites constructed *in vitro* have the expected properties for both endonuclease attack⁷ and *in vitro* recombination. In this process, the four more likely combinations (integration of fragment A, or B, in either direction) were observed suggesting that recombination occurred at random.

We show that a specific mammalian sequence can be transcribed from the coding strand in *E. coli* K12 under phage λ control. This is not unexpected in view of the known properties of the phage transcriptional systems¹⁶. The anti-termination properties of the *N* gene product make it likely that a number of sequences, including eukaryotic ones, can be transcribed under λ control, once integrated in appropriate phage early regions. This work provides an example of this, as a necessary step on the road to eukaryotic gene expression in *E. coli*.

Biohazards associated with the experiments described in this publication have been examined previously by the French National Control Committee. The experiments were carried out accordingly in P2-like conditions (in the NIH nomenclature). This work was supported by grants from CNRS, INSERM and DGRST in Paris, and by the FNRS in Geneva. One of us (F.R.) was supported by a short-term EMBO fellowship.

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Molecular characterisation of the tyrosine tRNA genes of yeast

MANY of the tRNA genes of yeast are observable genetically as nonsense suppressors¹. In particular, the set of unlinked suppressor loci *SUP2* to *SUP8* and *SUP11* have been shown to insert tyrosine at nonsense codons in yeast iso-1-cytochrome c^{2,3}. Direct biochemical evidence for the presence of suppressor tRNA in yeast strains bearing these suppressor genes has been obtained using *in vitro* translation systems^{4,5} and by the demonstration that a new minor tyrosine tRNA species with a suppressor anti-codon appears in a *SUP5* strain⁶. Although these

findings suggest that there are eight different loci in yeast which code for tRNA^{tyr}, only a single nucleotide sequence has been observed for tyrosine tRNA in wild-type yeast strains⁷. Thus it seems that all eight genes may share a common base sequence, at least in their structural regions. Because the yeast tRNA^{tyr} *SUP* genes occupy known positions on the genetic map⁸ and are complementary to a well characterised RNA molecule they are attractive candidates for molecular analysis. As a first step, we have labelled yeast tRNA^{tyr} with ¹²⁵I and have hybridised this probe to electrophoretically fractionated *Eco*RI fragments of yeast DNA; eight *Eco*RI fragments of different sizes hybridise to tRNA^{tyr}.

Figure 1 shows the molecular weight distribution of *Eco*RI-cleaved yeast DNA and documents the efficiency with which the DNA can be transferred from the gel to a nitrocellulose filter. In addition to the three prominent bands of molecular weight (mw) between 1 and 2 × 10⁶, which contain ribosomal cistrons, there is a continuous distribution of DNA sizes up to > 10⁷ mw (ref. 9). Comparison of the two curves in Fig. 1b shows that *Eco*RI fragments between 5 × 10⁵ and 5 × 10⁶ mw are transferred to nitrocellulose¹⁰ with 80% efficiency and that the transfer efficiency decreases significantly for DNA fragments larger than 5 × 10⁶ mw. The structure of the DNA banding pattern is retained remarkably well through the transfer process, as may be seen by comparing the gel stain pattern and filter autoradiogram (Fig. 1a).

DNA from yeast strain B596 (ref. 11) was *Eco*RI-restricted, electrophoresed on a 0.7% Agarose gel, and then transferred, as denatured DNA, to nitrocellulose filter strips¹⁰. After drying, the filter strips were hybridised to ¹²⁵I-labelled tRNA^{tyr}. The tRNA^{tyr} used for hybridisation was partially purified from total yeast tRNA by the acylation-BD cellulose procedure of Gillam *et al.*¹². The derivatised, ³H-tyrosine-aminoacylated tRNA^{tyr}

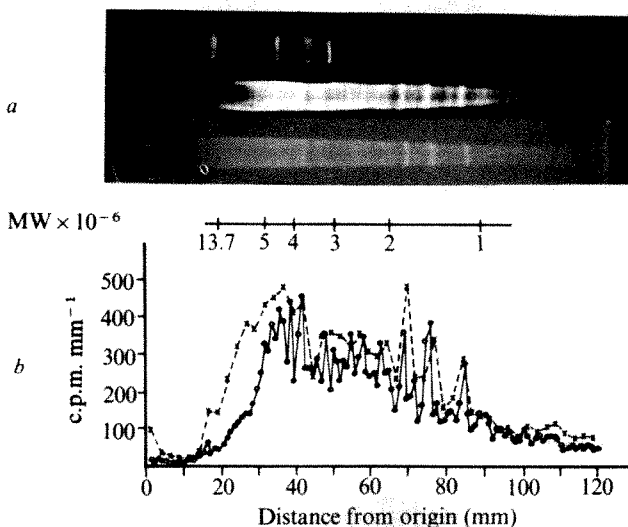


Fig. 1 Agarose gel fractionation of *Eco*RI-cleaved total yeast DNA. *a*, High molecular weight ¹⁴C-labelled yeast DNA was prepared by lysing glucanase-treated cells in sarkosyl and purifying the released DNA by velocity sedimentation through sucrose and isopycnic banding in CsCl. A limit *Eco*RI digest of this yeast was electrophoresed on a 0.7% Agarose slab gel, stained with ethidium bromide, and then transferred to nitrocellulose by the procedure of Southern^{10,11}. The top strip shows the banding pattern of *Eco*RI-cleaved DNA from bacteriophage λ , electrophoresed on the same gel. The middle strip shows an autoradiogram of the nitrocellulose strip containing the yeast *Eco*RI fragments. The bottom strip is a photograph of the yeast ethidium bromide staining pattern. *b*, On a parallel well of the same gel described in (*a*) an identical aliquot of *Eco*RI-cleaved yeast DNA was electrophoresed. The gel was sliced and the individual slices counted (broken line). The nitrocellulose strip whose autoradiogram is shown in (*a*) was also sliced and counted (solid line). The counting efficiency was the same for the gel and nitrocellulose filter slices so the two curves in (*b*) are a direct measure of the efficiency with which the DNA was transferred to the filter.

was eluted from BD cellulose in 1M NaCl, 11% ethanol. It was then iodinated with ^{125}I in the presence of peroxidase and H_2O_2 . In our hands this procedure gave material with a specific activity two orders of magnitude higher than we could achieve by *in vivo* labelling with ^{32}P . An autoradiogram of a hybridised filter strip is shown in Fig. 2a; eight discrete hybridisation bands are present.

To test conclusively whether tRNA^{tyr} molecules were responsible for the eight hybridisation bands observed in Fig. 2a, competition hybridisation was performed using cold competitor tRNA^{tyr} that had been highly purified by a procedure differing from that used to prepare tRNA^{tyr} for iodination. Specifically, the competitor was purified by sequential chromatography on BD cellulose, first as uncharged tRNA^{tyr} then as aminoacylated $\text{tyr-tRNA}^{\text{tyr}}$ (ref. 13); finally, the material was further purified by chromatography on Sepharose¹⁴ using a reverse ammonium sulphate gradient. Competition of the ^{125}I -tRNA hybridised to all eight bands is observable at an unlabelled tRNA^{tyr} concentration of $0.1 \mu\text{g ml}^{-1}$; competition is virtually complete at $1.0 \mu\text{g ml}^{-1}$. The estimated concentration of ^{125}I -labelled tRNA^{tyr} in the hybridisations was $0.1 \mu\text{g ml}^{-1}$. In subsequent experiments the same eight band pattern shown in Fig. 2a was obtained by hybridisation of yeast tRNA^{tyr} which had been purified to apparent homogeneity before labelling with ^{125}I . These data strongly suggest that each of the eight observed bands arises from hybridisation to a tRNA^{tyr} gene.

The molecular weights of the eight *Eco* RI fragments that hybridise to ^{125}I -labelled tRNA^{tyr} were estimated from their electrophoretic mobilities. Band positions on autoradiograms

Fig. 2 Hybridisation of ^{125}I -labelled tRNA^{tyr} to yeast *Eco* RI fragments. Iodinated tRNA^{tyr} with a specific activity of $>5 \times 10^7$ c.p.m. μg^{-1} was hybridised to DNA immobilised on nitrocellulose strips in 60% formamide, 1.15 M sodium acetate, pH 6 at 40°C for 3 h. The hybridisation conditions were similar to those used by Birnstiel¹⁶ for tRNA hybridisations. After extensive rinsing and RNase treatment, autoradiograms of the filter strips were recorded. Hybridisation was carried out in the presence of a, 0; b, $0.1 \mu\text{g ml}^{-1}$; and c, $1 \mu\text{g ml}^{-1}$ of purified, unlabelled tRNA^{tyr} .

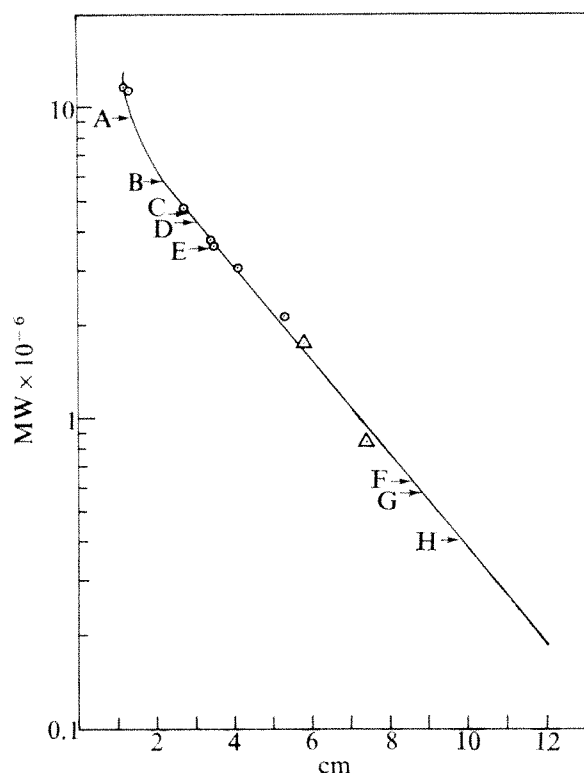
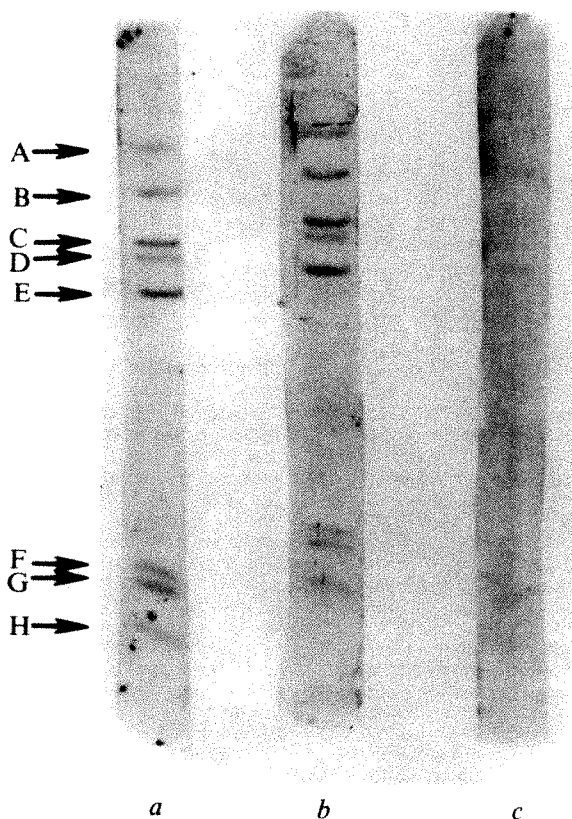


Fig. 3 Agarose gel mobilities of the yeast *Eco* RI fragments that hybridise to tRNA^{tyr} . The arrows show the positions of the eight hybridising fragments, A-H. λ *Eco* RI fragments, $\circ$; yeast rDNA fragments I and III, Δ . Molecular weights of the λ fragments are from ref. 15; values for the yeast rDNA fragments are calculated from data in ref. 16.

of the nitrocellulose filters were standardised with ^{14}C -labelled *Eco* RI fragments of bacteriophage λ (ref. 15) and also with yeast rDNA fragments I and III, which were located by hybridisation with ^{32}P -labelled 18 and 28S rRNA¹⁶. A plot from a typical gel is shown in Fig. 3. Approximate molecular weights for the hybridising fragments ($\times 10^3$) are as follows: A ≈ 9 ; B, 5.8; C, 4.5; D, 4.2; E, 3.5; F ≈ 0.6 ; G ≈ 0.6 ; H ≈ 0.4 .

Because the base sequence of yeast tRNA^{tyr} does not contain an *Eco* RI site, each DNA sequence that hybridises to this tRNA should lie within a single *Eco* RI fragment. Consequently, the number of hybridisation bands observed should equal the number of tRNA^{tyr} genes, which seems to be eight. The length of the *Eco* RI fragment which contains each of these eight genes will be determined by the nature of the chromosomal DNA sequences which lie to either side of the tRNA^{tyr} gene. Our results indicate that this neighbouring DNA has different characteristics for different tRNA genes, since the length of accompanying DNA varies from less than 1,000 to more than 12,000 base pairs for different restriction fragments which hybridise ^{125}I -tRNA^{tyr}.

An indication that one of the eight loci differs from the others within the tRNA gene is given by our hybridisation results (Fig. 2a and b). Band D contains noticeably less hybridised tyrosine tRNA than the more intense bands C and E on either side. We have observed band D to be consistently less intense than its immediate neighbours in a large number of experiments using DNA from several different haploid and diploid yeast strains. The data in Fig. 1b indicate that systematic loss of band intensity at low and high molecular weights is expected due to incomplete transfer of DNA to the nitrocellulose. This effect fails to explain the diminished intensity of band D, however, which is in a region of high, constant efficiency and is closely bracketed by more intense bands. Although differences in the number of tRNA^{tyr} genes at each locus remain a possible source of variable band intensity, the existing genetic data favour the hypothesis that each locus contains a

single copy¹⁷. To identify which suppressor gene corresponds to each of these bands, the procedures described here can be applied to DNA from yeast strains either deleted for particular suppressor loci or aneuploid for one of the relevant chromosomes. Further progress in the structural analysis of individual tRNA¹⁷ genes and the adjoining DNA sequences will depend on the successful molecular cloning of the *Eco* RI fragments that we have identified. The ¹²⁵I-tRNA probe described here should be well-suited to screening plaques or colonies for the presence of tDNA¹⁷ sequences in recombinant genomes.

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Nucleotide sequence of the yeast 5S ribosomal RNA gene and adjacent putative control regions

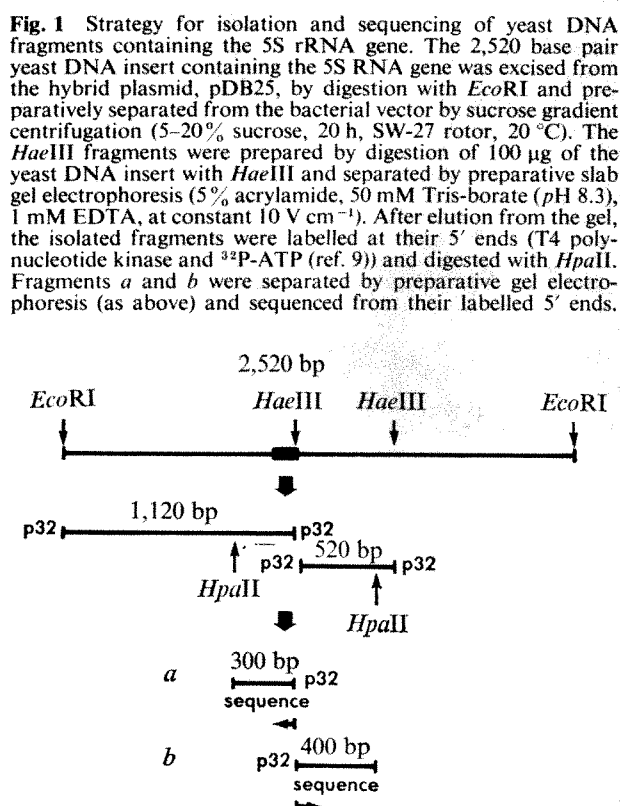
In bacteria, the sequence of several promoters has been determined and some of the features of their interaction with RNA polymerase, repressors and other regulatory molecules are beginning to be determined^{1,2}. In eukaryotes, however, the isolation and analysis of the DNA structures involved in the regulation of transcription has not yet been achieved. Recent advances in techniques for cloning eukaryotic DNA in bacterial plasmids³ have allowed the isolation of genetic segments whose structure and function can be studied in detail. Using these methods we have isolated a DNA fragment containing the 5S ribosomal RNA gene. The 5S RNA seems to be a primary transcript in yeast^{4–6}; therefore the flanking regions of the 5S gene should contain the putative promoter and termination regions. We report here the nucleotide sequence of *Saccharomyces cerevisiae* 5S DNA and these flanking regions.

Yeast DNA and the plasmid vector pSF2124 (ref. 7) were digested with *Eco*RI, ligated, and used to transform a derivative of *E. coli* K12, DG-75 (a F[–] derivative of DG73 ref. 8)). Recombinant clones were selected by ampicillin resistance and inability to produce colicin E1. Recombinants containing 5S rDNA were selected by hybridisation with purified yeast 5S rRNA. A clone (pDB25) containing

a 2,520 base pair yeast DNA fragment which hybridises with 5S rRNA was obtained.

The tentative position of the 5S rRNA gene within the 2,520 base pair fragment was determined by restriction mapping with endonucleases *Hae*III, *Alu*I and *Hpa*II and by hybridisation of the restriction fragments with ¹²⁵I-labelled yeast 5S rRNA (P.V. *et al.* in preparation). The sequence of 5S RNA of *S. cerevisiae*⁸ and of the related species, *S. carlsbergensis*⁹, predicts a *Hae*III site eight base pairs from the 3' end of the gene. Therefore as indicated in Fig. 1, we isolated the two *Hae*III fragments (1,120 and 520 base pairs, respectively) which encompass the 5S rRNA gene, labelled them at their 5' ends with γ -³²P-ATP and polynucleotide kinase, and digested them with *Hpa*II. Two fragments, each containing a complementary portion of the 5S RNA gene, of 300 (a) and 400 (b) base pairs were isolated by preparative gel electrophoresis and partially sequenced from their ³²P-labelled *Hae*III ends. DNA was sequenced by using the chemical cleavage technique developed by Maxam and Gilbert⁹. Figure 2 shows an autoradiogram of the electrophoretic separation of oligonucleotides resulting from base specific cleavages of fragments a and b. The sequence can be directly read from the autoradiogram as exemplified in the figure for the first 10 nucleotides. The experiment with fragment a (Fig. 2a) generated a sequence comprising 112 bases from the 5S rRNA gene and 61 bases from the region adjacent to the 5' end (termination region). An *Alu*I fragment of 160 base pairs containing this region was also sequenced (P.V. unpublished). The sequence of fragment b (Fig. 2b) furnished a sequence of 68 base pairs, 8 from the 5S gene and 60 from the region adjacent to its 3' end (initiation region). The complete sequence of the 5S RNA gene and adjacent regions is shown in Fig. 3. The sequence of other regions, comprising about 70%, of the 2,520 base pair fragment has been completed and will be reported elsewhere.

The sequence of most of the 5S RNA from *S. cerevisiae* has been reported by Miyazaki¹. However, a small region around the 35th position (from the 5' end) remained uncertain¹. The sequence we have determined for the 5S RNA



promoter region for *Xenopus* oocyte 5S RNA genes. There is no obvious relationship between the structures of this region and the region we have sequenced. The fundamental structural relationships which determine transcription promotion in eukaryotes are as yet difficult to perceive.

In contrast, at the end of the 5S gene there is a striking stretch of As in the coding strand (Fig. 3). We believe this to be a termination signal for RNA polymerase. Termination in other systems may be related to A-rich regions. For example, variable runs of Us have been found at the 3' end of 4S (ref. 11) and 6S (ref. 12) RNAs of λ phage, a small RNA from phage ϕ 80 (ref. 13) and the attenuator region of tryptophan mRNA of *E. coli*¹⁴. There is no evidence for transcription of this A region in yeast; however, transcription and rapid processing has not been eliminated. In eukaryotes, Brown and Brown¹⁵ have found a much shorter AT-rich region at the 5' end of the *Xenopus* 5S RNA gene and postulated the sequence TTTT AAAA as part of a transcription termination signal.

RNA polymerase binding and *in vitro* transcription experiments will be necessary to determine the role of these sequences in selective gene transcription.

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Promotor region for yeast 5S ribosomal RNA

We have sequenced the DNA region immediately preceding the structural gene for the 5S ribosomal RNA of yeast (*Saccharomyces cerevisiae*). Since the 5S RNA, a component of the larger ribosomal subunit, has a 5' triphosphate^{1,2}, this molecule is likely to be the direct product of the gene, unmodified by maturation at the 5' end. Thus the DNA sequence immediately before this region must contain the recognition sequences where the RNA polymerase binds to begin synthesis, that is, this sequence must represent a eukaryotic promoter.

T. D. Petes, P. Wensink and D. Botstein (unpublished) isolated a series of plasmids containing sheared DNA from a diploid yeast strain (A364a+D4, provided by L. H. Hartwell). Digestion of those containing yeast rDNA with *EcoRI* generated a map of the ribosomal area (T. D. Petes, L. Hereford and K.G.S., in preparation), shown in Fig. 1. The map was derived by noting the associations

between RI fragments in different clones carrying some but not all of the ribosomal repeat unit, and hybridisation of mature 5S, 5.8S, 18S and 28S rRNAs to the individual *EcoRI* fragments. Only fragment B, the second largest *EcoRI* fragment, 2,600 base-pairs long, hybridised to the 5S RNA. Sequential cleavage with *SmaI* (from *Serratia marcescens*) and *EcoRI* then oriented RI fragment B with respect to its neighbours.

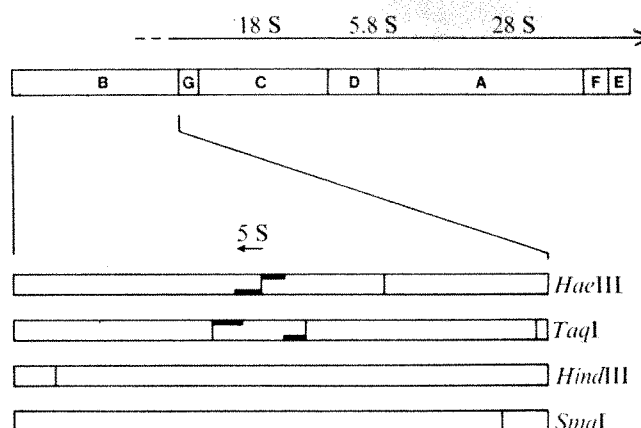
The nucleotide sequence of yeast 5S rRNA¹ suggested several restriction endonuclease sites in the structural gene, the one of primary interest being a *HaeIII* (from *Haemophilus aegyptius*) cut eight nucleotides from the 5' end of the RNA. To construct a restriction map of the *EcoRI* B fragment, we end-labelled it with polynucleotide kinase and λ -³²P-ATP, cut the fragment close to either one or the other end with *SmaI* or *HindIII* (from *Haemophilus influenzae*) (Fig. 1), and then examined partial digests of the resulting long, uniquely terminally labelled fragments with a series of restriction enzymes³. Sites for two which cleaved fragment B, *HaeIII* and *TaqI* (from *Thermus aquaticus*), are shown in Fig. 1.

We then sequenced⁴ the ends of all of the *HaeIII* fragments in order to identify which fragment junction lay within the 5S rRNA gene. From this *HaeIII* junction, we then sequenced out 100 bases before the gene, and on the other side, the gene itself (Fig. 1). Sequences at the extremities of this region were confirmed by sequencing towards the gene on the complementary strands from each of the two outlying *TaqI* cleavage sites. Figure 2 is an autoradiograph from one of these strands: a striking poly(dA) run that terminates the gene. Figure 3 shows the DNA sequence derived⁴ from analogous patterns on the four strands, and the corresponding 5S rRNA.

Our structure for 5S rDNA confirms most of the RNA sequences worked out by Miyazaki¹ for *S. cerevisiae* and by Hindley and Page⁵ for *S. carlsbergensis*, and generally agrees with one from *Torulopsis utilis*⁶. Except for two base changes (at positions 61 and 73) in *T. utilis*, the differences between the sequences are most likely to be minor errors in RNA sequencing: failures in ordering a series of pyrimidines within a single T₁ fragment (an extensive comparison of these sequences is in ref. 6).

The restriction maps and nucleotide sequences determine the direction of transcription of the 5S gene to be from right to left on the *EcoRI* B fragment, as shown, and thus

Fig. 1 Restriction endonuclease cleavage map of cloned *S. cerevisiae* rDNA. The order of fragments (A-G) released from the ribosomal repeat unit by *EcoRI* is shown at the top, and at the bottom, sites in fragment B for four other endonucleases. Arrows represent precursor RNAs, and thickened lines in the lower map indicate end-labelled DNA strands which were sequenced. (*EcoRI* map courtesy of T. D. Petes, L. Hereford, and K. G. Skryabin, in preparation).



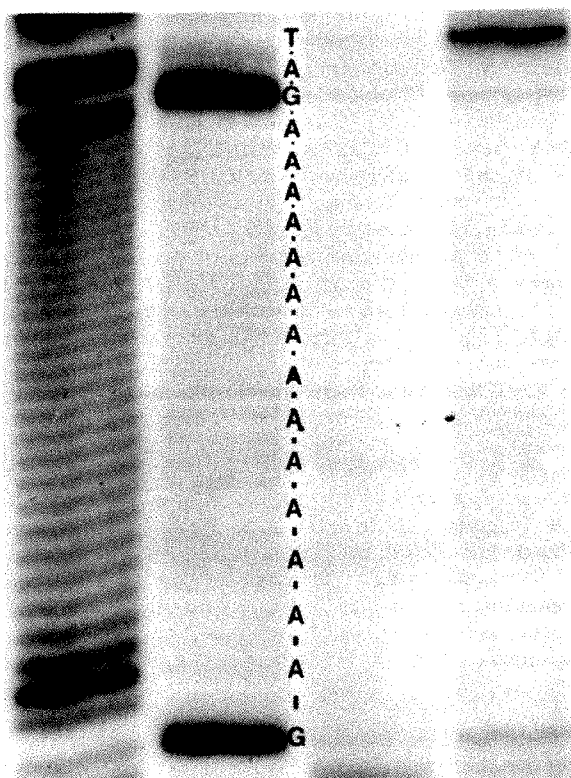


Fig. 2 Sequence pattern from one DNA strand in the region immediately following the structural gene for 5S rRNA. Partial, base-specific cleavage products extending from a labelled *TaqI* restriction endonuclease site towards the 5S gene (Fig. 1) are displayed. The specificity of the four DNA cleavage reactions employed was, from left to right, A > G, G, C, and C + T. Between the two guanines are 29 adenines, with every second one indicated on the autoradiograph. The centre of this poly(dA) region lies 142 bases from the startpoint of 5S RNA transcription, shown in Fig. 3. Valenzuela *et al.*¹⁰ (accompanying paper) find 21 adenines in this region in DNA cloned from another *S. cerevisiae* strain. To obtain the pattern displayed, *EcoRI* fragment B, isolated from the plasmid pY1R-B-3, was cleaved with *TaqI* and the products dephosphorylated with alkaline phosphatase, rephosphorylated with ³²P, and isolated on an 8% polyacrylamide slab gel¹. The *TaqI* fragment encompassing the 5S rRNA gene (Fig. 1) was then cleaved with *HaeIII*, and the end-labelled fragments separated on a second polyacrylamide gel and sequenced by partial, base-specific chemical degradation, gel electrophoresis, and autoradiography¹. The centre of the oligo-(dA) pattern in the autoradiograph is 68 bases from the labelled end of the fragment.

to be opposite in direction to the major 18S and 28S rRNA synthesis.

The region immediately to the left of the initial triphosphate in Fig. 3 must be the site for a yeast RNA polymerase. The 5S rDNA of animal cells is transcribed by RNA polymerase III (ref. 7); thus it is likely that this sequence contains the promoter for yeast polymerase III. Several elements in this yeast promoter sequence are curious (Fig. 4). There is a purine/pyrimidine bias in the 90 base pairs before the triphosphate, with 60 of 90 purines on the lower strand. There is no particular A-T/G-C bias. There is a triple partial repeat of an 18-base-pair-long sequence; 14 bases are repeated in the promoter and 11 bases early in the gene. This may be a remnant of the process that created this DNA region.

The promoter and the gene are flanked on the left and right with poly(dA-dT) rich regions almost serving to identify the gene itself (Fig. 3). Before the promoter there is a region of 16 A-T pairs; after the gene there is an oligo(dT) stretch 29 bases long. The sequence of Ts at the end of the gene is reminiscent of a number of bacterial termination sites (reviewed in ref. 8), but the poly (U) sequence, found on bacterial RNAs, would have to be removed.

If this yeast promoter is compared with the bacterial promoters known, no homologies are seen, nor might they be expected. In bacteria, the RNA polymerase interacts with a region of the DNA comprising some 40 bases before the beginning of RNA (reviewed in ref. 8). The polymerase protein also shields the first 18–20 bases that will be transcribed against attack with pancreatic DNase. The bacterial promoter's best defined region is the 'Pribnow box', a 7-base-pair-long approximate homology to the sequence TATPuATG centred on one turn of the DNA helix (8–11 bases) before the initiation point of the RNA chain. Another region defined by prokaryotic work is centred about 35 bases before the beginning of the messenger. Here there are some homologies, involving a GTTG sequence, and mutations in both the *lac* and the lambda promoters show that this region is important⁷. Furthermore, in bacteria the area around 60 nucleotides before the beginning of the messenger can be a site for positive control proteins. The CAP factor (the cyclic AMP binding protein that stimulates promoters such as *lac*, *gal* or *ara*) and the lambda repressor (in its *alter ego* of a positive control factor for its own synthesis⁹) bind there, often to palindromic sites.

The yeast sequence does not resemble any of the common

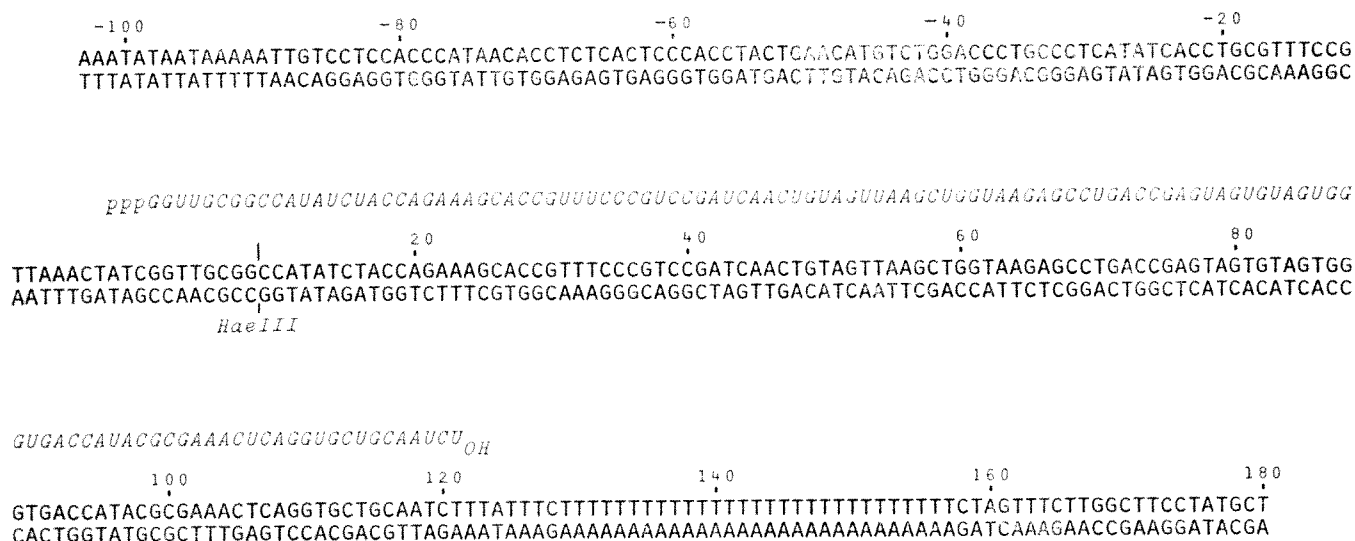


Fig. 3 Nucleotide sequences before, within, and beyond the *S. cerevisiae* gene for 5S rRNA.

pppG

CCTCCACCCATAACACCTCTCACTCCACCTACTGAACATGTCTGGACCCTGCCCTCATATCACCTGCGTTTCCGTTAACTATCG
GGAGGTGGGTATTGTGGAGAGTGAGGGTGGATGACTTGTACAGACCTGGGACGGGAGTATAGTGGACGCAAAGGCAATTTGATAGC

CCT C C CATA CACCT
GGA G G GTAT GTGGA

CCT C C CATA CACCT
GGA G G GTAT GTGGA

T A ATAA A T T A T A T A T A T T A T T T T TAAA TAT
A T TATT T A A T A T AT A TT TA A A T A A TATA T A AAA AATTT ATA

A A AA A A A A GAA A G GGA G A A A G G G AAA A G
GGAGG GGG A G GGAGAG GAGGG GGA GA G A AGA GGA GGGAG A AG GGA G AAAGG AA GA AG

Fig. 4 Nucleotide distribution in the promoter region for *S. cerevisiae* 5S rRNA. The guanosine triphosphate which initiates the RNA chain is shown above the DNA sequence at the extreme right. Underneath are shown a 14-nucleotide-pair repeating sequence and the distributions of A-T pairs and purine nucleotides in this region.

elements in the prokaryotic promoters; further exploration will be necessary to identify the critical nucleotides and regions with which the yeast polymerase interacts.

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Kinetics of rhodopsin at room temperature measured by picosecond spectroscopy

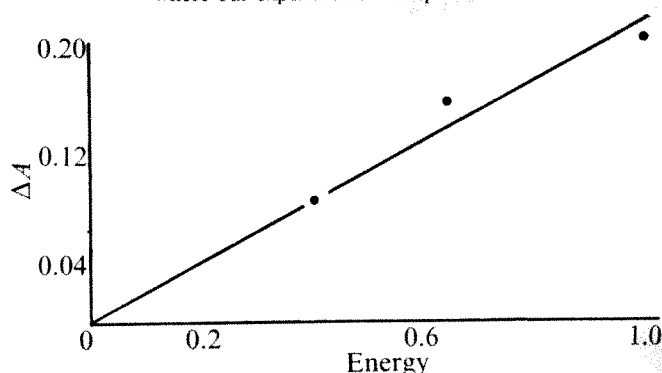
The photoinduced intermediates of the visual photoreceptor rhodopsin have been studied by optical spectroscopy in low temperature glasses and at room temperature. The primary changes in the absorption spectrum of rhodopsin were first observed at 77 K by Yoshizawa and Kito¹ then by Grellman *et al.*² and Yoshizawa and Wald³. The first intermediate observed, prelumirhodopsin (bathorhodopsin) exhibits a maximum at 545 nm and has been assigned to the primary isomerisation step of the rhodopsin polyene chromophore. The room temperature kinetics of formation and decay of an intermediate species absorbing at 560 nm were measured by Busch *et al.*⁴ using picosecond spectroscopy. This original work revealed that this species, assigned as prelumirhodopsin, was formed within 6×10^{-12} s and decayed with a lifetime of 3×10^{-8} s. Several technical innovations in picosecond spectroscopy⁵⁻⁷ now enable us to monitor spectra changes between 400 nm and 1,500 nm as a function of time with ± 0.02 absorbance unit resolution. We report here a complete difference spectrum for prelumirhodopsin

at room temperature mapped on a picosecond time scale. These studies show that the loss of rhodopsin and the rise of prelumirhodopsin are kinetically consistent. If care is taken to avoid photon saturation no 'ghost' species appear in the region of 400-440 nm as has been reported previously^{8,9}.

Bovine rhodopsin samples, solubilised in Ammonyx LO detergent, were prepared as described in ref. 10. The optically clear samples contained between 1 and 2×10^{-4} M rhodopsin (that is, 4-7 absorbance units per cm at 500 nm) and the absorbance ratios for 400 nm/500 nm were 0.20 ± 0.02 . For the experiments described, special care was taken to shield the sample from all spurious light emitted by the flashlamp or amplifier system, and all the experiments were conducted in the dark or under dim red light. Measurements were made in a 2-mm optical path length cell. We estimate that less than 15% of the sample in the optical path was bleached per excitation pulse. For each kinetic observation at a given wavelength, the sample was mixed between successive shots and completely replaced after five shots.

The experimental apparatus is described in detail in ref. 5. A single pulse is selected from the train of pulses generated by a Nd³⁺ glass laser emitting at 1,060 nm. The pulse is amplified and the frequency doubled to 530 nm. Thereafter it is split into two parts; one acting as the excitation pulse and the other inducing the interrogation continuum in a benzene or alcohol cell. This continuum is segmented into several spatially equidistant pulses by an echelon thereby providing the means for observing time resolved absorption spectra. These continuum pulses, which are too weak in intensity to cause any measurable excitation, are detected by a SIT vidicon and subsequently analysed, averaged, and displayed by a Nova computer. Care is taken to avoid dispersion and saturation effects by following the practice of timing the excitation pulse with

Fig. 1 Absorbance changes plotted against pulse energy. A straight line with an intercept at zero would indicate that no saturation occurred within this region of excitation pulse energy where our experiments were performed.



each wavelength and of making certain that the vidicon was used in its linear range.

Particular care was taken to eliminate photon saturation of the sample since rhodopsin, as most other highly sensitive biological molecules, can easily be saturated by an intense light pulse which may produce erroneous multiphoton events. Changes in ΔA were examined as a function of the laser pulse intensity as shown in Fig. 1 and data were used only when they fell within the linear range of this plot. An additional indication that the system is not saturating is shown in Fig. 2 in which the photoinduced ΔA increases proportionally with increasing sample concentration at constant excitation intensity.

Excitation of rhodopsin by a single, 6-ps 530-nm pulse resulted in the bleaching of the rhodopsin band and simultaneous formation of prelumirhodopsin. The entire difference spectrum, recorded 60 ps after excitation is plotted in Fig. 2. This spectrum is characterised by:

- (1) The bleached rhodopsin band at 430–510 nm with a maximum ΔA at 485 nm.
- (2) Formation of a band at 530–680 nm with a maximum ΔA at 580 nm.
- (3) An approximate isosbestic point at 525 nm.

This difference spectrum is in approximate agreement with the previously reported data for low temperature glasses in digitonin², rhodopsin in Ammonyx LO (ref. 10), and rhodopsin in intact retina¹¹. The kinetics indicate that the 485-nm band bleaches and the 580-band maximum arises within 6 ps as shown in Fig. 3.

There is one striking difference between the room temperature picosecond spectrum and those observed in low temperature glasses. The ratio of maximum and minimum ΔA in the difference spectrum observed at room temperature is 1:1. At low temperatures under a photo-stationary equilibrium, which is carefully established to exclude formation of isorhodopsin, this ratio is 2.5:1 or 3:1 (refs 10, 11). In addition the isosbestic point is located at 525 nm at room temperature versus 515 nm in low temperature glasses.

The source of this discrepancy is not clear. It is possible that temperature affects the band width and shape to some extent, however, it is more probable that at room temperature the excited rhodopsin decays not only to a prelumirhodopsin state but back to rhodopsin or other states via channels which are not open at low temperatures due to the rigidity of the species. To elucidate the operative mechanisms, we are investigating transient formation at various temperatures and attempting to determine quantum yields of formation of prelumirhodopsin as a function of temperature. It is interesting to note that a relative increase of the prelumirhodopsin band intensity at low temperatures would result in the shift of the isosbestic point to 515 nm,

Fig. 2 Difference spectrum observed 60 ps after excitation. The positive ΔA band is assigned to prelumirhodopsin while the $-\Delta A$ is due to the bleached rhodopsin. The reproducibility of each point is $\pm 0.02 \Delta A$ units. $\blacktriangle$, A 1.4/2 mm; $\bullet$, A 0.8/2 mm.

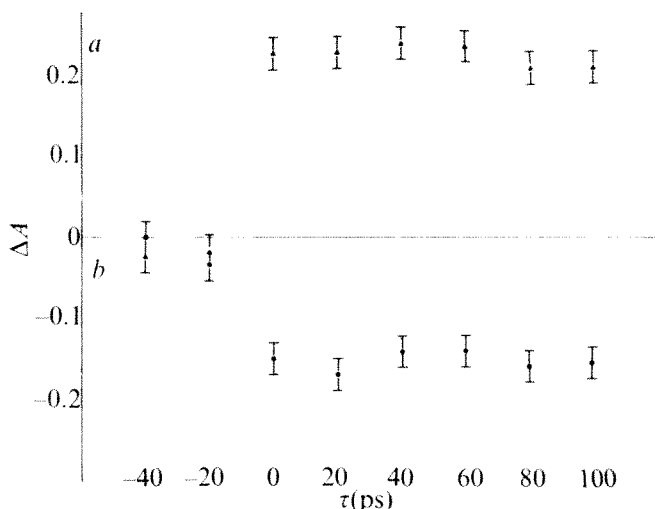
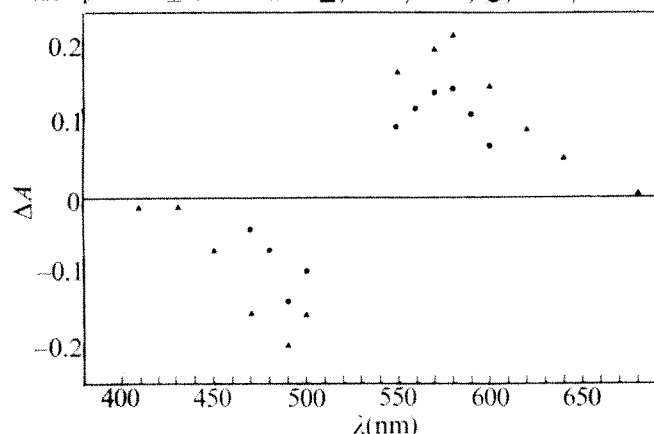


Fig. 3 *a*, Formation of the prelumirhodopsin band with a maximum at 580 nm induced by a 530-nm 6-ps pulse. $\blacktriangle$, 580 nm; $\bullet$, 480 nm. *b*, Depletion rate of the rhodopsin band at 480 nm after excitation with the same picosecond pulse. Similar kinetics are observed over the entire spectrum. Both figures represent an average of five kinetic records. The variance is given by the error bars.

which is observed at 77 K. This tends to support the above proposition that the 1:1 ratio observed at room temperature is probably due to active additional decay channels which deplete the excited state.

Rosenfeld and co-workers⁹ have reported the formation of isorhodopsin between 380 nm and 475 nm at 50 ns. Bensasson *et al.*⁸ reported a similar absorption occurring at 50 ns which was attributed to a new absorption due to prelumirhodopsin. As noted above, we have not observed formation of any new species between 410 nm and 430 nm between 6 ps and 100 ps nor do we have evidence for the early formation of hypsorhodopsin, the species observed by Yoshizawa¹² at liquid helium temperatures.

In view of the general agreement of our spectrum (Fig. 2) with regard to the shape, width, maxima, and kinetics (Fig. 3) with the previously published spectra for prelumirhodopsin at low temperatures, we assign the species we observe at room temperature to prelumirhodopsin. We conclude that within our time resolution of 6 ps, the only species formed at room temperature during the first 100 ps is prelumirhodopsin.

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matters arising

Transition probability and cell-cycle initiation in yeast

SHILO *et al.*¹ have suggested that the transition probability model, devised to explain the regulation of proliferation of mammalian cells², can also be applied to yeast. They propose that traverse of an early G1 event called 'start'³ is probabilistic and is the rate-limiting step of the cell cycle. This suggestion arose from their investigation of the kinetics of bud emergence in cells released from a block at 'start', imposed either by using a strain conditionally defective in *cdc 25* or by using the mating hormone α factor. After release from a block at

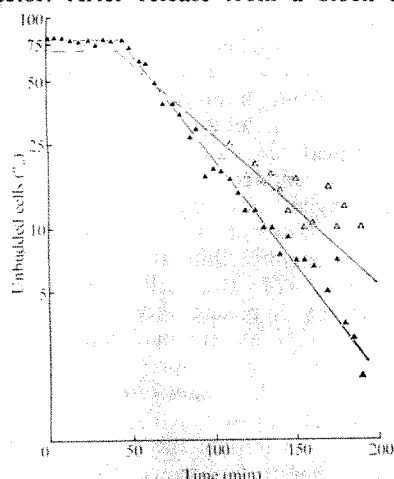


Fig. 1 The kinetics of bud emergence according to Fig. 1 in ref. 1, replotted. The proportion of unbudded cells (ordinate) is plotted on a logarithmic scale against time after release of the block (abscissa). ▲, strain 352/1 (*cdc 25*) after release of temperature block; △, same strain after release of α factor block.

'start', the proportion of unbudded cells seemed to fall exponentially, indicative of a probabilistic traverse of 'start'. Unless the proportion of unbudded cells is followed to a low level, however, it is difficult to distinguish between an exponential line and the lines derived from other distributions of cell generation times, such as reciprocal normal (A. E. Wheals, personal communication). Of the six semi-logarithmic plots given by Shilo *et al.*¹ only one continues below a level of 10% of the starting value, and in this case (Fig. 1 in ref. 1) the line curves gradually downwards. There is also scatter of the points around the fitted exponential lines, particularly in Figs 2 and 4. Therefore the evidence in

favour of a probabilistic traverse of 'start' is not convincing.

Shilo *et al.* also suggest that the kinetics of bud emergence after release from a block at 'start' are the same as in a steady state culture, indicating that 'start' is the rate-limiting step in the cell cycle. This interpretation is only possible if the kinetics of bud emergence after release from the block are due solely to the traverse of 'start' and not to physiological recovery from the block. In the examples given this is not in fact so; although it is claimed that bud emergence shows similar first-order kinetics whether the block is imposed by α factor or by *cdc 25*, this only seems to be so because the data are on linear plots. If the data are replotted semi-logarithmically (Fig. 1), the lines can be seen to be different, the half life of the unbudded state for cells after release from the α factor block being 42 min, whereas after release from the *cdc 25* block, the half-life is 29.5 min. Corresponding transition probabilities (P values—see ref. 2 for definition) are 0.63 and 0.76 h^{-1} . Clearly the nature of the block at 'start' is affecting the kinetics of bud emergence, which nullifies any comparison with a steady state culture.

Interpretation of these experiments is further complicated by the use of *cdc 25*. Mutants in this gene are unusual as they stop growth at the restrictive temperature, unlike mutants in most other *cdc* genes⁴. For this reason Johnston *et al.*⁵ consider it likely that *cdc 25* is affected in intermediary or macromolecular metabolism and that its arrest before 'start' is only a secondary pleiotropic effect. If this is so then the experiment plotted in Fig. 2 only determines the kinetics of recovery from this block in metabolism and is not informative about the rate limiting step of the cell cycle.

Note added in proof: Variability in event traverse is not evidence for its rate-limiting nature unless it can be established that event traverse is probabilistic. Thus, knowledge of the exact kinetics of cycle initiation (see reply by Shilo *et al.*) is essential for determining whether 'start' is the rate-limiting step of the yeast cell cycle.

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SHILO *et al.*¹ claim that their data support the concept of transition probability² in the yeast cell-cycle. However, evidence that fails to distinguish between two alternative hypotheses cannot be regarded as evidence in support of one of them. There are at least two contrasting models to account for the transition from an expandable part of the cell-cycle to a determined phase. The transition probability hypothesis argues that it occurs at random, there being a constant probability per unit time that a cell will undergo the transition^{2,3}. The cell size hypothesis suggests that cells monitor their own size and as they grow they undergo the transition when they have reached a critical cell size⁴.

Rate of entry into the budded phase is reported as showing first-order kinetics¹, but the measurements of the fraction of unbudded cells were not continued below 2%. Above these values there is virtually no measurable difference between first-order kinetics (α -curve), a lognormal distribution⁵ and a reciprocal normal distribution⁶ as can most clearly be seen when the same data are plotted equally successfully in quite different ways, for example HeLa cells^{2,7,8} and *Euglena*^{2,9,10}. Whereas first-order kinetics is consistent with the transition probability hypothesis, both lognormal and reciprocal normal distributions are consistent with critical cell-size hypotheses and the rate of entry could be interpreted as reflecting the heterogeneity in the size of the unbudded cells and the variation in time needed to grow to a critical cell size. Concomitant measurements of size were not made. Where this has been done, attainment of a critical cell size has been shown to be an important factor in the initiation of division in, for example, *Physarum polycephalum*¹¹, *Stentor*¹², *Amoeba*¹³, *Schizosaccharomyces pombe*¹⁴, and *Saccharomyces cerevisiae* (refs 15,16) and Wheals's unpublished data.

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B. SHILO, V. SHILO and SIMCHEN reply
 —We believe that the asynchronous kinetics of cell-cycle initiation are a genuine characteristic of the 'start' process¹ and not an artifact of the methods used to arrest the cells². As shown in Table 1, five different haploid strains were arrested before 'start' by a variety of methods, including temperature blocks of the temperature-sensitive 'start' mutations *cdc 25*, *cdc 28* (ref. 3), *cdc 35* (L. H. Hartwell, personal communication) and *tra 3* (ref. 4), α -hormone arrest of *a* cells, and stationary phase arrest of cells at a high titre (4×10^8 cells per ml). In some of the treatments, cells were arrested for different lengths of time. In all cases, the half life ($t_{1/2}$) of the unbudded state after release from the block was in the range of 42 ± 13 min.

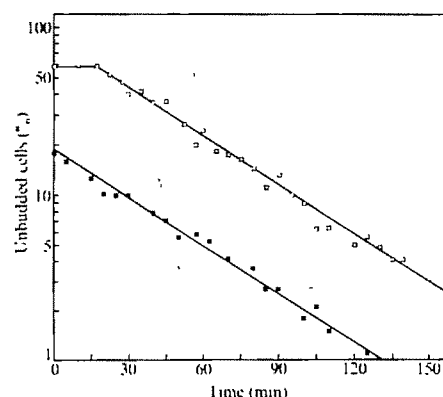


Fig. 1 Kinetics of bud initiation in an exponentially growing culture and following release from α -hormone block. The haploid strain A364A (ref. 3) is of mating type *a* and contains the markers *ade1*, *ade2*, *uia1*, *gal1-4*, *his7-1*, *lys2-2* and *trp1-2*. The cells were exponentially grown in YEPD medium¹ at 25 °C. They were slightly sonicated¹, diluted to 1.7×10^6 cells per ml and plated on YEPD medium solidified with 2% agar, at 25 °C. After plating, the % of unbudded cells in each sample was determined by counting at least 400 cells under the microscope. In the second experiment A364A cells were plated on YEPD solid medium following release from a 2.5-h block by a mating hormone¹. ■, Exponentially growing A364A cells after plating. □, A364A cells following release from an α -hormone block.

Table 1 Rates of release from blocks at 'start' and at a later stage (*cdc24*)

Strain	Mutation	Block type	Block period (h)	$t_{1/2}$ (min)
A364A	wild type	α -hormone	2.3, 2.5	32, 31
		Stationary	4, 4.8×10^8 cells per ml	42, 52
352/1	<i>cdc25</i>	36 °C	2, 3.3, 3.5	55, 43, 29
		36 °C	6	41
343	<i>cdc28</i>	α -hormone	2.5	39, 42, 50
		38 °C	3.8	41
353	<i>cdc 35</i>	α -hormone	2.5	38, 43
358	<i>tra3</i>	36 °C	3.3, 3.5	53, 38
337	<i>cdc24</i>	36 °C	2.8, 3, 3.3, 3.3, 4, 4.5	46, 53, 53, 10, 7, 10, 4, 9, 9

All cultures were grown in YEPD medium¹ at 25 °C prior to arrest and after release from the block. Each $t_{1/2}$ value was obtained in a different experiment.

This value is five times larger than the mean $t_{1/2}$ value obtained following release from a *cdc 24* (ref. 3) block, which comes after 'start'. The variation in $t_{1/2}$ values indicates that the physiological state of the cells, the block itself and the genetic background of the strain may have some effect upon the kinetics of cell-cycle initiation. But, these effects seem only to modify, not to generate, the asynchrony in cycle initiation. In extreme cases these effects result in physiologically deprived, or even non-viable cells, which remain unbudded for a very long time. We therefore feel that data on the initiation of the last 2% of the cells would not give reliable data on the kinetics of the entire population, as has been suggested^{2,3}.

To avoid the effects of the method of arrest on cell-cycle initiation, we have conducted an experiment in which budding was monitored in an exponentially growing culture of wild-type cells. It is known that a fraction of the unbudded cells in a growing culture is located between cytokinesis and 'start'. According to the "transition probability" hypothesis⁴ this fraction is expected to decline exponentially on plating on solid medium. This was indeed observed (Fig. 1), with the frequency of unbudded cells going down from 18% to 1%, according to first-order kinetics. Moreover, the rate-constant was very similar to that obtained with the same strain following removal of α -hormone block (Fig. 1). This experiment strongly supports the suggestion that first-order kinetics are a genuine characteristic of cell-cycle initiation.

The suggestion that *cdc 25* cells are arrested at 'start' as a secondary effect of a pleiotropic mutation⁵ is based only on the finding that they do not show any cellular growth at the restrictive temperature⁷. The latter observation can be interpreted differently, however. If the resting stage is just before 'start', we would expect cells to maintain a balanced physiological state as well as a constant cell size when arrested at 'start'. Following this rationale the *cdc 25* temperature block

is at the natural resting stage of the cell. Even if our interpretation of the *cdc 25* mutation is not accepted, it should be pointed out that the temperature-shift experiment (ref. 1, Fig. 2) was repeated with strains carrying mutations in either *cdc 28* or *tra 3*, that were arrested at the restrictive temperature, and with *cdc 25* cells that were arrested by α -hormone. Similar results were obtained in all experiments.

It has also been suggested that the asynchronous rate of cycle initiation results from different periods required by cells in the culture in order to reach a critical size⁶. The "transition probability" model⁴ and the "critical cell size" hypothesis⁶ are not mutually exclusive, however. We have mentioned¹ the possibility that the physiological condition of the cell could affect 'start' by imposing threshold requirements such as minimal size. Indeed, a recent report⁷ presents evidence suggesting that *Saccharomyces cerevisiae* cells must reach a minimal size in order to initiate the cycle at 'start'. We suggest, however, that heterogeneity in population size alone cannot be the reason for the asynchronous rate of cell-cycle initiation. After a cell reaches the critical size, initiation of the cycle is not an immediate consequence, but seems to depend on a random event. This suggestion is supported by the following experimental findings. (1) On release from a temperature block, the first buddings of the mutant *cdc 24* on solid medium appear synchronously (ref 1, Fig. 1). The second buddings are asynchronous, however, following similar kinetics to that of cell-cycle initiation with a $t_{1/2}$ value of 31 min. The theory of critical size cannot explain asynchronous budding of cells which have already produced at least one bud (and apparently reached the 'critical size'). (2) 'Start' arrest of *cdc 28* cells at the restrictive temperature, or *a* cells arrest by α -hormone, do not block cell growth, in contrast to *cdc 25* temperature arrest⁷. Similar kinetics of cycle initiation were observed in all three cases, however (Table 1).

In conclusion, we feel that the experimental data given here and in our previous publication¹ support the "transition probability" model⁶ for cell-cycle initiation, though admittedly additional variables may have to be taken into account. Discussions about the exact kinetics of cycle initiation, however, should not obliterate our main conclusion about 'start'¹, namely that it is the rate-limiting step of the yeast cell cycle.

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Fission-track dating of pumice from the KBS Tuff, East Rudolf, Kenya

HURFORD, Gleadow and Naeser¹ claim to have fission-track dating results supporting the controversial 2.61-Myr value for the age of the KBS Tuff in East Rudolf, Kenya as determined by K-Ar dating²⁻⁴. The fission-track age does not, however, contribute substantially to solving this controversy, particularly since the authors¹ have not drawn attention to two important points, namely the error limits of the age and the current uncertainty about the spontaneous fission constant of uranium-238.

First, the quoted error of about 3% seems unrealistically small and probably represents only precision. The authors should give also the age accuracy which is necessary for comparing different radiometric ages. Second, many fission-track specialists no longer use the $6.85 \times 10^{-17} \text{ yr}^{-1}$ value, but now use as the decay constant $8.46 \times 10^{-17} \text{ yr}^{-1}$; there are good reasons for this preference⁵. If this higher value for the decay constant is used, the fission-track age of the pumice in the KBS tuff recalculates to 1.98 Myr, which would lend support to the K-Ar age measured by Curtis *et al.*³. For stratigraphic use of fission-track ages one has to be critically aware of, and draw attention to, the present uncertainty of the spontaneous fission constant of uranium-238.

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NAESER, HURFORD AND GLEADOW
REPLY—We feel that the age we reported is a reasonable estimate for the age of the zircons separated from the pumice lumps in the KBS Tuff¹. If our age is wrong, it is wrong for reasons other than our choice of the decay constant² for the spontaneous fission of ²³⁸U. Two possible sources of error in this age are geologic in origin:

(1) The samples were collected in a sedimentary sequence. In this type of occurrence, contamination by detrital zircons is always possible and, in fact, is quite common. One advantage of the fission-track dating method is that the age of single crystals can be determined. A detrital zircon having an age greater than 10 Myr can easily be excluded from the population being dated. The problem occurs when the contaminating zircons are only a little older than the zircons being dated. The statistics of individual grains are such that a zircon having an age of 6 Myr would be included in the data because it cannot be reasonably separated from the rest of the population. In this case, however, five different determinations were made by three different individuals, and it seems highly unlikely that all three would choose the same relative numbers of contaminating grains. For this to happen, the detrital and pyrogenic zircons would have to be present in equal proportions, and the age of the detrital zircons could not be much greater than about 3 Myr.

(2) These zircons contained many small needle-like inclusions. Some of these could possibly have been counted as tracks, and this would result in an older age. As was true for the first source of error, this type of counting error would have to have been made by all three laboratories to the same extent.

Wagner² has questioned our choice of a decay constant, $\lambda_F = 6.85 \times 10^{-17} \text{ yr}^{-1}$ (ref. 3). When it is used in conjunction with the fission track glass standards of the U.S. National Bureau of Standards⁴, we get the best agreement with the K-Ar ages of co-existing minerals and we use it for this reason. This agreement has been found for minerals such as zircon, apatite, and sphene, as well as natural glasses that have not suffered track annealing. Figure 1 shows the results of 34 zircon fission-track ages plotted against the average K-Ar age of one or more minerals from the same rock. These ages are all from volcanic or sub-

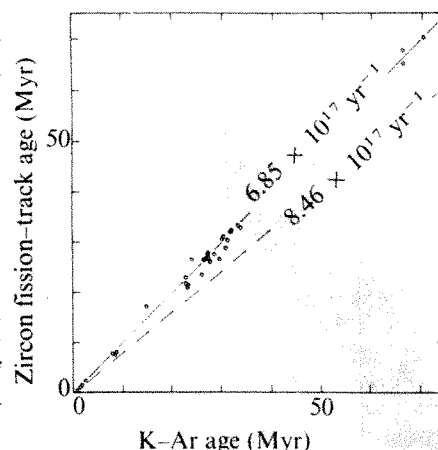


Fig. 1 Zircon fission-track ages and the average K-Ar ages of minerals from volcanic and subvolcanic rocks. λ_F values $6.85 \times 10^{-17} \text{ yr}^{-1}$ (solid line) and $8.46 \times 10^{-17} \text{ yr}^{-1}$ (broken line).

volcanic rocks in which annealing should be absent or minimal. Alternatively we could have chosen an empirical method⁵ to calculate the ages of the KBS Tuff zircons. This method is independent of λ_F and neutron-dose calibration, simply requiring a number of samples from well-dated rocks. Had we chosen this method, our results on the zircons from the KBS Tuff would have been the same.

Wagner has also questioned our statistics². The precision of a single fission-track age determination is not equal to that of a K-Ar age, and probably never will be, but five separate determinations can produce a mean that has a reasonably small error associated with it. The accuracy of any age can only be guessed at, in that we do not know the true age of any geologic sample. We can only strive for the best agreement with K-Ar and the other dating methods.

We therefore think that our age of 2.4 Myr is a reasonable estimate of the age of zircons separated from the pumice lumps present in the KBS Tuff. If Wagner feels that we must use $\lambda_F = 8.4 \times 10^{-17} \text{ yr}^{-1}$, then he must show us where we have a corresponding 20% error in our method that compensates for our choice of decay constant and that will account for our close agreement with K-Ar ages.

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Southern oscillation index and atmospheric carbon dioxide

BACASTOW¹ claims that the Southern Oscillation (SO) modulates atmospheric carbon dioxide, which may be true, but was not proven by his article.

Bacastow compared a SO index with both carbon dioxide concentrations and its time derivative. In his Fig. 1 carbon dioxide concentrations were smoothed in an unspecified manner. Taking the derivative is equivalent to applying a high pass filter to the data and results therefore depend on the original smoothing. With different forms of smoothing applied to each series the phase relations will be affected.

The period of data used in the study was from 1958 to 1974, which includes the period 1960–1965 when the SO was not operating effectively. Trenberth² shows that, in the early 1960s, the correlations of pressure between Easter Island and Darwin—required by the SO mechanism to be negative—were in fact positive. He further shows that the SO operates only on a time scale of 2–10 yr and mainly in the 3–6-yr range. Marked quasi-biennial fluctuations occur at Darwin, but not Apia, Tahiti, or Easter Island in the South Pacific. The quasi-biennial oscillation and the SO are not apparently the same thing, although they may interact at times. Furthermore, lags of up to 1 yr are involved in the opposite changes at Darwin and Easter Island³.

Further consideration should therefore be given to the choice of a SO index. Trenberth suggests that a simple index involving only Darwin and Tahiti would

Table 1 Southern Oscillation index correlation with CO₂ data

Derivative anomaly	SO index	Smoothing	Correlation maximum	Anomaly lag (month)	Standard deviation
South Pole	$P_{EAS} - P_{DAR}$	12 month running mean	-0.64	6	0.34
Mauna Loa	$P_{EAS} - P_{DAR}$	12 month running mean	-0.55	2½	0.28
South Pole	$P_{UAS} - P_{DAR}$	Spline	-0.70	6	0.36
Mauna Loa	$P_{EAS} - P_{DAR}$	Spline	-0.56	2½	0.31
South Pole	SO1	3 month running mean	-0.60	4	0.26
South Pole	SO2	3 month running mean	-0.59	4	0.24

P_{EAS} and P_{DAR} , seasonal pressure anomalies at Easter Island and Darwin respectively.

be best since these stations have the highest negative correlation (-0.76 for yearly values for 1944–73) and are almost exactly out of phase. This could be used to define lag relationships elsewhere. Seasonal, rather than monthly data should be used.

To find such an index, we note that an exact inverse correlation between Darwin and Tahiti, using their standard deviations gives

$$P_{Darwin} = -1.26 P_{Tahiti} \quad (1)$$

where P is the seasonal pressure anomaly. Since part of the mass compensation from the Indonesian centre of action of the SO occurs south of Australia and New Zealand as well as in the South Pacific³, greater weight should be given to the pressure at Darwin in a SO index (see Fig. 6 of ref. 3). A suitable weighted index, based on covariances, is

$$SO1 = P_{Tahiti} - 1.2 P_{Darwin} \quad (2)$$

As only two stations are included, some noise would be present, but is measured by

$$SON = 1.26 P_{Tahiti} + P_{Darwin} \quad (3)$$

which from (1), has a zero value if the SO is operating.

An index that combines (2) and (3) is

$$SO2 = \frac{P_{Tahiti} - 1.2 P_{Darwin}}{1 + |SON|} \quad (4)$$

I suggest that this index may be more useful than any previously used.

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BACASTOW REPLIES—Trenberth is correct in pointing out that different filtering of two time series will, in general, affect their correlation–time lag relationship. I do not believe, however, that this consideration is important in judging the existence of a connection between the Southern Oscillation (SO) and the atmospheric CO₂ level. If a Southern Oscillation Index is generated by smoothing the Easter Island–Darwin, Australia, pressure differences by the procedure applied to the CO₂ data, the cross correlation functions of this index with the time derivatives of the CO₂ anomalies (see Table 1 for summary) are essentially the same as found for these pressure differences smoothed by a 12 month running mean.

The mechanism behind the SO is poorly understood, so the best index to represent it is unclear. The indices suggested by Trenberth (calculated from data in ref. 1) are qualitatively quite similar to the Easter Island–Darwin Index, except for the obvious difference in degree of smoothing (Fig. 1). Pressure anomalies were smoothed by a 3-month running mean, in accordance with Trenberth's suggestion that seasonal data be used. Cross correlation functions of SO1 and SO2 with the CO₂ anomaly derivative at the South Pole (Table 1) are very similar to those found for the Easter Island–Darwin Index, except that the lag of the anomaly is about 2 months less relative to the Tahiti–Darwin indices, as would be expected from the analysis of Trenberth².

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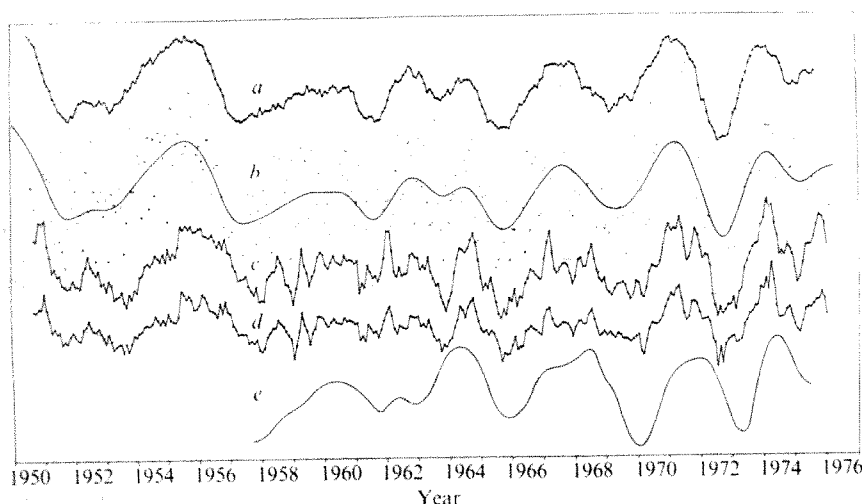


Fig. 1 Southern Oscillation indices. *a*, Differences in monthly average pressure between Easter Island and Darwin, Australia, smoothed by a 12-month running mean (which also removes the seasonal effect). *b*, Pressure differences above with the seasonal effect removed. The curve is a spline obtained by the procedure used to find the CO₂ anomalies, and, accordingly, the stiffness of the spline was adjusted by eye. The seasonal effects were calculated by an average over identical months of the difference between the pressures and the spline. *c*, SO1. The seasonal effects at Tahiti and Darwin were determined by an average over identical months of the monthly average pressure records (since 1950) and removed from each record by subtraction. Each record was then smoothed by a 3-month running mean and SO1 calculated. *d*, SO2. *e*, Inverted time derivative of the South Pole anomaly, for comparison.

reviews

Disaster book

Peter J. Smith

Forces of Nature. Edited by Sir Vivian Fuchs. Pp. 303. (Thames and Hudson: London, 1977.) £9.50.

PERHAPS UNWITTINGLY, this book plays a small part in a curious paradox. For the past few years the world economy has been in recession, human institutions have been under threat as seldom before, and the general feeling has been one of gloom and despondency. But as if that were not enough, the same period has seen a remarkable upsurge in the publication of books about those macroscopic forces of nature which can, and do, contribute to recession, destroy human institutions and give rise to gloom, not to mention real hardship. The level of interest in earthquakes, volcanic eruptions, drought, floods, avalanches, tornadoes and forthcoming ice ages has recently reached a new height and shows no sign of falling. It is almost as if we were trying subconsciously to diminish the relative force of human mismanagement by pointing to greater events beyond our control.

But if so, we are doomed to failure, for what Sir Vivian's book tells us time and again is that in the face of disruptive natural forces human folly is only magnified. The ancient city of Antioch was repeatedly destroyed by earthquakes and equally repeatedly rebuilt on the same site with similar materials and no more effective rules—a self-defeating activity which even the most highly developed nations have been unable to avoid. In 1963 the Vaiont reservoir in Italy overflowed violently with the loss of 2,600 lives, because no-one had read the lessons plainly visible in the local history of landslides. In Switzerland the obvious dangers of avalanche are often ignored even by experienced mountaineers. In countries where drought is endemic, populations grow unchecked, making each disaster more devastating than the last. And so on.

Under the circumstances it is perhaps understandable that Sir Vivian should take a dim view of human capability. Of all the forces of nature, he suggests in his introduction, "none can be controlled by human agency. Even if our growing knowledge was to make this possible, it is doubtful if we have, or could acquire, the wisdom to handle

them for the general good . . . It may well be wiser to accept the broad interplay of natural forces, always learning better how to use them or to protect ourselves from them." Not everyone would agree with that, regarding it as overly defeatist, for the reference to protection reminds us that man is not entirely incapable of fighting nature. From the Tennessee Valley Authority (for all its faults), through splitting wedges in the Alps and the sea wall at Skegness, to the person who decides that Denver offers a safer life than Los Angeles, there is ample evidence that nature needed not always win.

In *Forces of Nature*, each chapter of which is written by "an expert in his field", man's attitudes and responses to natural phenomena are given more than usual attention, although firmly set against a background of scientific explanation. The blend of science and sociology works well, leading to a nicely balanced text that should have wide appeal. What ultimately makes the book, however, is less the text than the remarkable collec-

tion of photographs (researched by Sarah Waters), some in black-and-white but many in colour.

And therein lies something slightly unnerving. For the truth is that in the hands of a brilliant photographer, and with a magnificent standard of reproduction, the consequences of the most devastating natural disaster may be recorded as something of astonishing beauty. The funnel of a tornado crossing Oklahoma, a parched and drought-cracked river bed in Kenya, rooftops projecting from a major flood near Brisbane, the distortion of a flood-swept railway line in the Netherlands, and molten lava from Etna advancing towards civilisation, must all have represented absolute despair to someone; and yet on the page they make pictures as enticing in their way as a *Hay Wain* or a *Fighting Temeraire*. It's all vaguely disconcerting, but makes for a fascinating book. □

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Time problems in land processes

Geomorphology and Time. By J. B. Thornes and D. Brunsden. Pp. 208. (Methuen: London, 1977.) Hardback £6; paperback £3.45.

WHAT George Poulett Scrope wrote in 1858, that "the leading idea which is present in all our researches, and which accompanies every fresh observation, the sound which to the ear of the student of Nature seems continually echoed from every part of her works, is—Time! . . . Time! . . . Time!", could be equally well be applied to studies today. It was the geologist who discredited the rigid biblical timescale and extended the overall limits of earthly time from under 6,000 years to over 4,000 Myr. Geomorphologists joined the geological bandwagon with one hand on the leading rein. Quite early they began to be interested in rates of processes, and eventually acquired an assortment of morphological measurements and crude timescales

for the effects of denudation and deposition on landforms.

Being concerned with present processes, geomorphology has contemporary utility, and the hope has always been that the present would also provide a key to the past. This is of course, true of the action of processes and untrue of landscapes. In any event, time-independent processes cannot exist. So time is a necessary ingredient of geomorphological research and must be considered with other essential features such as lithology, process and spatial variability, all of which impinge on problems of scientific observation, model-building and interpretations of the temporal occurrence of events.

The authors of this book elucidate these ideas on time in a lively introduction which treads warily around many pitfalls and will stimulate much thought. For example, we are told that velocity is expressed as distance moved in unit time (L/T). This seems reason-

ably applicable to large deep rivers, but I wonder if, in fact, in steep shallow channels with rocky, pot-holed beds, where some water movement is uphill, geomorphologists do measure the true distance travelled by a water particle in a given length between two successive points. Among the many interesting topics mentioned in these introductory pages are relaxation time and feedback in energy systems.

The following chapter discusses at some length the criteria that Earth scientists use to obtain a chronology. Relative morphological vertical position, extent of drainage patterns, stratigraphy, mineral and organic content, weathering, artefacts, radiation, tree rings and lichenometry all find a welcome place, but thermoluminescence might perhaps have been better replaced by palaeomagnetism. The discussion then proceeds to the measurement of variables in time, the analysis of temporal data, and the rates of operation of geomorphological processes. In these the authors rightly consider that awareness of techniques will make the reader more careful in designing experiments for the collection of time-based data and more aware of the inadequate nature of much of the published data on measured rates of processes.

Collagen biochemistry and biophysics

Biochemistry of Collagen. Edited by G. N. Ramachandram and A. H. Reddi. Pp. xv+536. (Plenum: New York and London, 1976.) \$59.40.

UNTIL some 30 years ago, collagen was of interest mainly to industrial chemists concerned with the properties of leather, glue and gelatine. Since then, there has been a rapid expansion of information concerning collagen structure, metabolism and function. This book, as the title implies, covers the biochemistry of collagen but also contains chapters more properly labelled biophysics.

The chapter by Piez collates the information available on the primary structure of collagen polypeptide chains. Also discussed are the sequences involved in the unique post-translational modifications, particularly hydroxylation and crosslinking. There are brief descriptions of the methods of isolation of the different collagens and of the polypeptide fragments to which the usual methods of sequencing have been applied.

The next three chapters of the text provide information on the various types of models concerned with the way that events change in time. The qualitative temporal variety leads on to the quantitative deterministic type and so to the less certain stochastic models of form evolution. Not surprisingly, as geomorphologists deal with area realities, there follows a discussion on the setting of events or points into a framework wherein spatial characteristics or patterns evolve. A summary of space, and time and a long bibliography of mainly post-1960 literature, form a suitable conclusion.

In brief, this neat and well-illustrated book provides at a reasonable price a commendable introduction to recent conceptual advances in time-related factors in geomorphology. Geomorphologists may have far to go and some time to wait before their recordings on rates of process become truly reliable but this excellent summary will provide students with a clear view of the time-scale aspect of present achievements.

Robert P. Beckinsale

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The chapter by Ramachandran and Ramakrishnan provides a general account of the early X-ray diffraction studies which led to the formulation of a triple-helical structure for collagen, together with a more detailed consideration of recent modifications of this basic structure. The chapter on synthetic polypeptide models is largely redundant, much of the material being covered by other authors.

The molecular packing of collagen monomers in fibrils is the subject of a chapter by Miller who discusses critically the methods available for studying this packing, particularly X-ray diffraction and electron-microscopy, and the results obtained by these techniques. More tentatively, he discusses the possible models which can be derived from the data available.

The complex processes by which the collagen molecule is synthesised are dealt with by Prockop and his colleagues. After a short section on collagen mRNA, the manifold post-translational modifications which the primary structure of collagen undergoes are discussed. These include hydroxylation of lysine and proline, and glycosylation of hydroxylysine. Also discussed is the final intracellular process which involves the assembly of three polypeptide chains and the subsequent triple helix formation before the

molecule is secreted. The final section is a stimulating discussion of the regulation of these intracellular processes by which the wide range of chemically different collagens found in the body are achieved.

After secretion the completed molecule is cleaved to form a molecule which can form a fibril by a process of self-assembly. The stabilisation of the fibrillar structure by covalent crosslinks is discussed in a chapter by Tanzer. This deals with the structure and mechanism of formation of these crosslinks.

The enzyme mammalian collagenase, one of several proteases involved in collagen degradation, is the subject of a very useful chapter by Gross. The most interesting section is that concerned with the regulation of collagenase activity and how it is synchronised in time and space with collagen biosynthesis.

The chapter by Timpl on immunological studies is concerned with the diversity and localisation of antigenic determinants on the collagen molecule. There are useful sections on the use of specific antisera to detect anticollagen antibodies and also as tools to study the structure and metabolism of collagen. There is a section on the use of fluorescent collagen antibodies to localise and follow the temporal changes in the appearance of the different genetically determined collagen.

The molecular basis of diseases affecting collagen is dealt with in an excellent chapter by Lapière and Nugens. This contains two tables of data on the cellular and molecular basis of collagen defects, and on the techniques applied to define collagen pathology. Various diseases of connective tissue, both inherited and acquired, are discussed in the light of current knowledge of collagen structure and metabolism.

The chapter by Reddi is somewhat disappointing. It covers rather sketchily an area of research concerning the intimate involvement of collagen in cell differentiation and organogenesis. This is an important topic requiring a more detailed and critical review than that provided by this chapter.

There is evidence of a considerable gap between the completion of the manuscript and final production of the book (compensated in some chapters by addenda). Nevertheless this is a reasonably up-to-date account of the current status of collagen research which should be useful to workers in a wide variety of disciplines.

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Ecological parasitology

Ecological Aspects of Parasitology. Edited by C. R. Kennedy. Pp. x+474. (North-Holland: Amsterdam and Oxford, 1976.) Dfl 145; \$57.95.

PARASITISM is an ecological term, and parasitology is essentially an ecological study. Some aspects of parasite ecology have received extensive coverage for many years, especially those related to qualitative and quantitative epidemiology, where the unit of study is the infection rather than the parasite.

The stated object of this book is to remedy the situation whereby some aspects have received little attention. The aspects chosen in the first section concern the parasite outside the host: dispersal (C. R. Kennedy), host location (A. J. MacInnis) and host selection (J. C. Holmes); entry into the host (D. W. T. Crompton), feeding (C. Arme), interaction between parasites (D. Halvorsen), and host responses to parasitic infection (D. Wakelin). The bulk of the book is in part two. Here, "Habitat" is seen as the immediate surroundings of the parasite. There are two chapters on fish: skin (G. C. Kearn) and gills (C. H. Fernando and C. Hanek); and the remaining chapters deal with the special features of parasitic life in different mammalian or avian organs and cells. In the final chapter, R. M. Anderson discusses the interaction of host and parasite populations.

With 24 authors covering so wide a field, consistency and uniformity are not to be expected or even desired. If the intellectual verbosity of chapter one were maintained throughout, the book would be hard indeed to read. Particularly noteworthy is yet another definition of parasitism, first credited with elegant beauty, then, mercifully, discredited as being possibly applicable to all organisms!

The rest of the book is clear and stimulating. The only truly unique feature of the parasitic habitat is the specific antagonistic reaction that it produces. This aspect is given extensive treatment. D. R. Arthur describes how amputee mice, unable to groom, are subject to massive infestations of lice; D. Wakelin gives a lucid comparison between the immune processes of invertebrates and vertebrates, and A. D. Befus and R. B. Podesta describe the special immunological features of the intestine.

Most other aspects of parasite ecology have clear parallels in free living organisms. Useful comparisons are made but unfortunately not ex-

panded with both sessile and planktonic marine animals. More such comparisons could have been enlightening.

The great value of this book lies in the descriptions of various organs from a parasitological viewpoint. Almost every author points out areas of extensive ignorance in his field, and it is clear that too few physiologists, biochemists and physicians have a clear idea of what ecology is about. The book will surely be useful as a

stimulant for discussion and further investigation. It is a little disappointing that in so extensive a series of reviews there is a sad lack of coordinating thought. Perhaps, though, that is what the book will help to produce.

R. W. Ashford

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Electron theory of small molecules

Introduction to the Electron Theory of Small Molecules. By A. C. Hurley. Pp. 329. £12; \$26.25. *Electron Correlation in Small Molecules.* By A. C. Hurley. Pp. 276. £10.50; \$23. (Academic: London and New York, 1977.)

THESE two books are perhaps meant to be read together. The author states in his preface to the *Introduction* that the book consists of the "earlier and simpler chapters of what was originally conceived of as a comprehensive one-volume work". As such, the *Introduction* is to provide what the author calls a "royal road from basic quantum mechanics to the various theories which are now yielding such detailed information on molecular interactions", some of which are discussed in the *Electron Correlation* volume.

The *Introduction* provides an account of the basic theory of potential energy curves and surfaces, an outline of the variational method for constructing approximate electronic wave functions and a short account of the (generalised) virial and Hellmann-Feynmann theorems. It also provides a brief, but self-contained account of molecular symmetry and quite extended accounts of the determinantal approach and of molecular orbitals and the Hartree-Fock method. The accounts are presented chiefly in the context of results for diatomic molecules (although small polyatomics occasionally receive a passing mention) and a chapter each is devoted to detailed consideration of the hydrogen ion and of the hydrogen molecule.

The book is regrettably something of a disappointment to the reviewer, if for no other reason than that it seems so old-fashioned. A suspicion of this may be gleaned from the first few pages in which it becomes clear that the author is going to use c.g.s. units; but it is re-inforced, for example, by his treatment of spin-coupling and of methods for solving the SCF equations,

for which he gives no references more recent than 1967. Indeed, if one looks through the text one gains the impression that the phrase "recent work" should really be synonymous with "ten-year-old work". This criticism should not, of course, be construed as a call for "trendiness", but there is work in the past five years in the areas quoted above (and indeed in other areas in the book) to which the attention of the reader should be called even in an introductory book like this.

As one would expect from an author of Dr Hurley's distinction, there are a lot of good things in the *Introduction*. There is, for example, a splendid and precise account of Koopmans' theorem, a very nice heuristic account of the various cusp conditions in the wave function, some very helpful material on the continuous groups in the section on symmetry, a brief and clear account of the RKR method, and many more. There are, however, some funny slips. The author says, for example, that in the quadratic approximation to the potential, the equation for nuclear vibrational motion in the diatomic is the simple harmonic oscillator equation. But the student who has read his Eyring, Walter and Kimball *Quantum Chemistry* knows that this is just not true. The equation (2.60) from which he begins his account of the generalised Hellmann-Feynmann theorem seems to be simply wrong at an elementary level (see the author's own original paper, *Proc. R. Soc. A* **226**, 170 or Appendix 1 in Pople *et al. J. Chem. Phys.* **49**, 2960; 1968), and in view of Dr Hurley's contributions to the foundations of this part of the subject this (if the reviewer is not mistaken) is quite incredible. Be that as it may, the discussion in this part of the book has nothing like the subtlety or power of the discussion of the same material offered by Epstein in his recent book (*The Variation Method in Quantum Chemistry*, Academic: London, 1974); and Epstein's book is not referred to at all.

At a somewhat different level, the author's discussion of natural orbitals

(continued on p654)

will strike the initiated reader as very eccentric; and I do not think it will help the beginner. The index of the book also seems to have its eccentricities. If one looks up "charge density" for example, one gets referred to two pages on neither of which is the concept defined or anything other than incidentally mentioned. One does not get referred to the section on p303, which has charge density in its heading, which is perhaps just as well because the section contains no mention at all of the idea.

The book cannot therefore be recommended unreservedly as an introduction to the field but it is nevertheless a book that repays careful reading and should be useful to those teaching advanced undergraduate and graduates in the subject, if only because there is not really another book in this area.

Electron Correlation consists of just two chapters, one entitled "First Order CI and VB methods" and the other (about 200 pages) "The Correlation Problem". Again, the emphasis is on diatomics but polyatomics get a better look in than in the *Introduction*. The underlying and unifying theme of the book seems to be designing wave functions for molecules which correctly describe their dissociation into fragments. In the first chapter the author takes a rather eclectic view of CI and VB, and the reader looking for detailed accounts of the current state of the art in these methods will be disappointed. In fact most of the chapter is devoted to a discussion of symmetry principles, of a particular multi-configuration SCF method and of an introduction to the theory of separated electron pairs. The second chapter is devoted almost

entirely to pair theories culminating in an account of the coupled pair approximation treated in a manner equivalent to Cizek's treatment. After a short discussion of the requirements necessary to obtain chemical accuracy using such methods the chapter ends with a brief account of putting r_{12} explicitly into the wave function particularly by the *trans*-correlated method.

Once more there are lots of good things in this book. There is a splendid account of natural spin-orbitals and the convergence of the CI expansion, and of the difficulties in iterative pseudo-natural orbital approaches. There is a very clear and precise account of the deficiencies inherent in particular classes of wave functions constructed from strongly orthogonal groups. Indeed, the whole account of development of pair theories is clear, coherent and precise, and extremely well illustrated by results from actual calculations.

Again it does seem to lack references to more contemporary work: obvious omissions are the work of Meyer and that of Ahlrichs and his group in the pair function field. One would also expect to see more references to recent work in the CI and VB approaches. The result is that the book must strike the informed reader as a somewhat unbalanced account of the field.

The reviewer's reservations here are, however, only minor. *Electron Correlation* is a book that many practising quantum chemists will surely want to own.

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Ice Age, ancient and modern

The Ice Age: Past and Present. By Brian S. John. Pp. 254. (Collins: London, 1977.) £4.95.

ADDING to the rapidly expanding recent literature on glaciers, Ice Ages and such topics, Brian John has produced a useful non-technical volume that is designed to appeal to "the person who knows very little about ice and glaciers and even less about the Ice Age". In 235 pages it covers a great deal of ground, from how glacier ice is formed to its effects on the Earth's surface, from ancient Ice Ages to speculative comments on future climatic change. Inevitably the treatment becomes somewhat superficial in places, as when the botany of the Ice Age is surveyed in just 12 pages; but on the whole it

succeeds very well in dealing with such a huge canvas. Moreover, it is a soundly based book: on geomorphological and geological aspects, John knows his stuff, and therefore unlike many popular or semi-popular accounts, the text is reliable and up-to-date.

The arrangement of material is clear and logical. The first half of the book looks at the nature and origins of glaciers, their effects on landforms through erosion and deposition, the landscapes associated with both continental and alpine types of glaciation, and the work of glacial meltwater in washing out and transporting debris away from the edge of the ice. There follow short chapters on ice in a periglacial environment, touching briefly on permafrost, frost weathering, patterned ground, pingos and other ground-ice features, and on sea ice, dealing with ice shelves, pack ice and icebergs.

The second half of the book deals mainly with Ice Ages. Sensibly, for

this type of book, John begins with the recent historical past, discussing the effects and significance of the "Little Ice Age", before delving further into the past to consider the Pleistocene period and then the pre-Pleistocene glaciations, as well as the innumerable theories of the causes of Ice Ages. There is an outline of the effects of glaciation on land and sealevels; a minor criticism here is that *non*-glacial causes of changes in level tend to be ignored so that one gets the erroneous impression, for instance, that the sinking of the North Sea basin is somehow bound up with glaciation. Three chapters provide brief summaries of Ice Age plants, animals and man, and the book concludes with a discussion of what the future may hold in store—when will the present interglacial come to an end, how will it end, and what impact is man having on the world's glaciers, intentionally or unintentionally?

Throughout, John tries to write in a simple non-technical style. On the whole he probably succeeds in making the subject matter intelligible to the non-specialist, but often at the risk of over-simplification. At times, however, he lets his imagination run away with him. Such statements as "You don't have to ask an Eskimo whether we are living in the middle of an Ice Age", or "Like the Irish navvies of Victorian times, the most active glaciers tend to be the most efficient, and where they have plenty of excess energy they achieve prodigious feats of erosion and transportation", are probably acceptable in a book of this sort, but some other passages draw rather far-fetched similes. Did he really have to write that "Unstable glaciers cannot find relief from the stresses of life by weeping or getting drunk", or that "Pingos prefer to live in groups . . ." (they are described as "lovable creatures" because they tend to cluster together)?

The book is well produced with few errors. An amusing slip is 'subsiding' instead of 'subsiding' on p230. The 32 half-tone plates are mostly of high quality (the ERTS imagery of Skeidararsandur is an exception), and the line-drawings are clear. The latter, oddly, are not numbered and not specifically referred to in the text. There is a short list of books for further reading, and an index. At £4.95, the book represents reasonable value for money; it ought to attract the non-specialist reader and it will certainly stimulate the intelligent sixth-former (advanced school) student to find out more about ice and Ice Ages.

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nature

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Dismantling the firebreak

RESEARCH and development of tactical nuclear weapons has been conducted for many years now with relatively little public debate against a background of even less public information. So it would be unfortunate if the recent revelation of budgetary proposals for 'enhanced radiation' devices to be built by the US Energy Research and Development Administration were to be seen only as a step, and maybe not a very big one at that, towards a new type of weapon. For the whole question of tactical nuclear weapons, regardless of their mixture of blast and radiation content, deserves a much wider airing.

Tactical nuclear weapons have yields of the order of tons rather than the kilotons or megatons of strategic devices. They can be fired from tanks as shells; and as miniaturisation proceeds they are more easily adapted to mortars or even suitcases. Enhanced-radiation/reduced-blast tactical devices would carry the added attraction for the field commander that the area bombarded would not be so devastated and full of radioactive debris as to be inaccessible subsequently to his own troops. So an enhanced radiation device would be ideal for land battles where territorial advantage counted.

Not so ideal, however, is the way recent developments in nuclear weapons have been closing the gap between

conventional explosives and strategic nuclear weapons. Until the late 1960s there was a very clear firebreak between the limited damage that chemistry could inflict and the devastation that physics could wreak. Soldiers could be entrusted with conventional weapons, but only presidents could launch nuclear devices. Then to bridge this gap came tactical weapons that were scaled down strategic weapons.

Now the talk is of devices that cause even less damage but kill more, and they are beginning to look distinctly like biological weapons. Advocates of the new devices still see firebreaks to prevent a limited confrontation from escalating into an all-out nuclear war, but this is a very optimistic point of view and assumes that belligerents will recognise each other's devices for what they are, have no wish to respond more aggressively and have the right weapons available to permit response in kind. It's a tenuous line of argument to say the least, and many will find it totally unconvincing.

In the past 25 years an immense amount of thought has been devoted to the possibilities of restricting strategic armaments. The need now is for some equally intensive studies to be made of ways of averting the escalation which new weapons technology is making progressively more likely. □

Dissecting the crisis

RECENTLY the European Commission, the EEC's administrative-cum-executive arm, sponsored a colloquium in Brussels entitled 'Crisis of science in European societies?' Everyone who attended seemed to take it for granted that the answer to the question is 'yes'. But though that may be right, the crisis borne of the modern problems of the bomb, the environment, energy, resources and changing social attitudes has been around for some years now. And crises, like lost tempers, should be over quickly if they are not outward signs of something more serious.

The Brussels symposium could hardly have been said to have come to grips with this deeper problem, beyond revealing that there are different opinions and judgments about what should be done. For some this may be suf-

ficient—a symbolic meeting of cultures, an agreeable renewal of old friendships, may be all they sought from such a meeting. They might even see it as pretentious to claim that gatherings like these could go further, though just such hopes were being entertained.

But there is meanwhile a real and deepening problem for many scientists. It involves falling funds, less research and fewer researchers. And it highlights what is perhaps the most serious problem to be faced: that scientists, unlike most other discernible groups, do not seem able to organise themselves to talk plainly to governments. Nor for that matter does the decision-making system encourage such organisation. Amiable gatherings in Brussels are a poor substitute for hard talking nearer home. □

Too late not to proliferate?

David Fishlock examines the background to the burgeoning international trade in nuclear technology

DR Glenn Seaborg, the chemist who with E. M. McMillan first isolated plutonium, delivered a paper on "the plutonium economy of the future" in Santa Fe in 1970. It was an important paper; Seaborg, as chairman of the US Atomic Energy Commission, was forecasting that by 1990 one-quarter of the energy used by the USA could come from plutonium. By then, he said, the nation would have accumulated a plutonium stockpile from its light water reactors worth (at 1970 prices) about \$6,000 million—twice as much as the US investor-owned utilities had spent on fuel in 1969.

The paper attracted little attention. The campaign against nuclear energy in the US, although well under way, was almost wholly engaged in attacking the intrinsic safety of nuclear reactors. Proliferation of nuclear materials, although a subject of deep concern to a great many governments, was not a public issue, much less a target of anti-nuclear campaigners. Neither were nuclear weapons or nuclear materials then seen as serious targets for international terrorists.

By the mid-1970s, however, the anti-nuclear campaigners badly needed a new target. Although they had succeeded in slowing the pace of licensing and construction of reactors, they had failed completely to make out a case against them as a potential hazard. Not a single nuclear plant had been shut down because it was emitting, or was considered liable to emit, dangerous amounts of radiation.

Plutonium, and plans to refine and recycle it, and to use it to fuel more advanced types of reactor, offered a tempting new target. The very name of the element, its "fiendishly toxic properties" as one US physicist had once described them, and its immense potential as an explosive offered clear advantages in arousing public fears. Overnight the 'plutonium economy' became a catchphrase depicting a world unfit for human beings.

Three events

Three events contributed importantly to the widespread fears now aroused about the plutonium economy and its implications. The first, in May 1974, was the explosion by India of a plutonium device it euphemistically referred to as a "peaceful nuclear explosive". This was a profound shock

for those nations which for nearly 20 years had been striving for international controls on the use of nuclear technologies. In public India had declared repeatedly that it would never make a nuclear weapon. So, in spite of the fact that India had been opposed from the outset to any idea of international control and safeguards through the UN's International Atomic Energy Agency (IAEA), such nations as the US and Canada had provided India with nuclear technology and know-how, sometimes on very generous terms. Under the Colombo Aid programme Canada provided an NRX research reactor free from international safeguards. The Indians used this reactor to transmute natural uranium into plutonium.

The second event occurred about a year later, in the summer of 1975, when after some months of publicity the West German Government signed a contract with Brazil to provide that country with virtually a ready-made nuclear industry, including the technology to separate plutonium and to enrich uranium. It was the biggest nuclear export contract any country had ever won. But the US Government had already turned down Brazil's requests for enrichment and reprocessing, not least because Brazil refused to sign the 1969 Non-Proliferation Treaty (NPT).

The furore over the German-Brazilian contract also highlighted a number of other nuclear contracts under negotiation, notably contracts under which France planned to provide Pakistan and South Korea with reprocessing plants. Together with the Indian explosions, these negotiations prompted the US Government to bring together for the first time the seven leading nuclear supplier nations at a secret meeting in London to try to find more effective controls for the transfer of 'sensitive nuclear technologies'. These were then defined as the technologies of reprocessing and enrichment, both of which could be adapted to yield fissile materials, and the production of heavy water.

The third event of importance in establishing the plutonium economy as a public issue was the publication in September 1976 of the Sixth Report of the Royal Commission on Environmental Pollution, under the chairmanship of Sir Brian Flowers. In spite of the fact that Flowers had never once raised the matter as a part-time mem-

ber of the UK Atomic Energy Authority, this report expressed deep concern with the idea of any plutonium falling into the hands of terrorists. If the stability of our society continued to deteriorate, it suggested, proliferation of the sources and movements of plutonium could begin to pose a serious hazard.

Meanwhile the US Government had been abruptly awakened to the fact that it was fast losing control of technologies it had sought to control—for example, through its patronage of the IAEA and its domination of the enrichment and reactor markets—since the end of the Second World War. It searched desperately for fresh safeguards against proliferation. Dr Henry Kissinger, as Secretary of State, enthusiastically endorsed an IAEA idea for regional fuel cycle centres operating under international management and safeguards to serve such areas as Latin America and the Middle East with 'sensitive technologies'. A major study by the IAEA soon disclosed that while the regional concept was politically impractical—it was difficult to conceive of a project or a site that would serve Brazil, Argentina and Chile, for instance, or Egypt, Israel and Iran—the multinational reprocessing centre was a serious proposition. Where the centre was located was of small importance since transport costs varied little with distance. But economies of scale made the economic advantages of large plants attractive to any nation with purely commercial aims. The IAEA concluded that ideally a multinational reprocessing centre would be set up by five or seven nations, one or more of which would be able to provide the technology and organise the financing of plants expected to cost of the order of \$1,000 million. Such a centre would justify full-time surveillance by IAEA inspectors to ensure that no illegal diversion of plutonium took place.

Interest waned

Promising as the outcome of this study proved to be, the interest of the US Government had waned long before its formal presentation at the nuclear conference in Salzburg next month. At best it could be a medium-term solution, for such centres would need at least a decade to organise and bring into service. Even the technologically simpler problem of organising international plutonium storage might take a decade to bring about. The US was urgently searching for tighter controls against proliferation that could be implemented here and now.

Its response came finally in a statement on 7 April in which President Carter formally declared a moratorium on one of the sensitive technologies,

reprocessing. In future the US would store the highly radioactive fuel from its light water reactors. The president took care at this time to stress that this was domestic US policy for controlling proliferation, and that it recognised that other nations with no indigenous uranium might not be so willing to forego the uranium and plutonium locked up in spent fuel.

During the month that followed two further aspects of US anti-proliferation policy emerged. One was that in order to try to persuade other nations to follow the US policy, America was going to expand her uranium enrichment capacity while still keeping this activity firmly under government control. The implication was that it was prepared to supply enriched uranium from its own reserves, enriched to the low level required in existing thermal reactors, and moreover would be prepared to store unprocessed spent fuel for customers.

What also emerged, in a statement from Dr Joseph Nye, a senior State Department official concerned with anti-proliferation, was that the US would bring pressure to bear on foreign governments to follow its policy by refusing permission to reprocess, either at home or elsewhere, fuel which had previously been enriched in the US. In practice, this meant most of the enriched fuel extant in the West.

This ban could be implemented under the bilateral agreements covering enrichment of the original fuel. Where customers, said Dr Nye, were in special difficulties from lack of storage capacity, and plants might have to be shut down, the US Government would be prepared to consider special dispensation. But licences to re-export the fuel for reprocessing would be granted only in cases of "clear need". There could be no question of a general dispensation of the kind necessary to support the 10-year contracts under negotiation between the Japanese electricity industry and Britain and France.

These statements evoked widespread and sometimes vehement criticism from countries which would be immediately affected, notably those having no indigenous uranium but busy laying foundations for plutonium-fuelled fast breeder reactors to limit their dependence on uranium imports. One specific criticism levelled against the US was that for reasons of domestic expediency it was prepared to renounce one sensitive technology while expanding another, enrichment; and using, moreover, a technology—the gas centrifuge—which only a few years before it had argued would pose a serious threat to the control of proliferation. More generally it was asked what guarantees a nation could have for placing its faith in the US, whose own motives and intentions regarding fast reactors are uncertain.

Developing nations, whatever their nuclear aspirations, indignantly attacked what they saw as a rich nation's attempt to debar them from a valuable technology. The USSR, which has its own uranium reserves and also reprocesses spent fuel for its satellite nations, keeping the plutonium in Russia, has indicated that it will have nothing to do with President Carter's proposals. The policy could only hamper its own well-developed plans for fast reactors.

As with Russia, Britain has longstanding plans for safeguarding the future of its energy supplies once North Sea reserves run out by burning plutonium in fast reactors. Recycled in this way, Britain's existing stockpile of uranium has an energy content exceeding that of proven coal reserves. Britain also has plans to use its knowledge of the difficult technology of reprocessing to develop an international service, under IAEA safeguards, for nations which would prefer not to embark on this technology, at least at this stage. These plans could be severely hampered by US refusal to grant re-export licences for reprocessing to nations whose fuel has been enriched in the US.

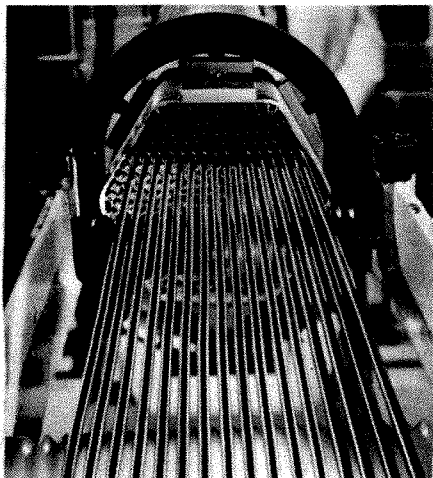
Dr David Owen, the UK Foreign Secretary, in the fullest public response yet by any nation, on 10 May pointed out bluntly some of the weaknesses, as Britain sees it, of President Carter's anti-proliferation policy. He emphasised that Britain remained firmly committed to the principles of non-proliferation. But an effective anti-proliferation policy "must go hand-in-hand with a viable energy strategy", he said. He pointed to routes to nuclear explosives which the US policy would apparently leave uncontrolled. And he further implied that President Carter, far from improving relations, has seriously worsened them in this highly sensitive area of international affairs.

Continued proliferation?

A more realistic approach to the problem might be to assume that nuclear weapons will continue to proliferate. Although their attractions to terrorists have probably been greatly exaggerated, the attraction to governments remains undisputed. And governments would find it far easier to assemble the resources required to make credible nuclear explosives. In 1945 only one nation possessed these explosives. Through the McMahon Act the US debarred Britain, a partner in the Manhattan Project, from further participation in the technology. The UK Government, convinced that it needed the technology for broad reasons of national security, promptly embarked on its own nuclear programme. The USSR and France took similar decisions; and later China and India. An impressive 104 nations have signed the NPT, eschewing nuclear explosives; but another 30 have not, among them some with well-developed nuclear plans.

No moratorium on all, still less on parts, of the nuclear technologies by states already possessing nuclear weapons is credible as a way of stopping one of those 30 nations if it is determined, as Britain once was, to acquire the Bomb. It could even encourage it in such a quest. Neither need these nations try to buy large nuclear power stations costing around \$1,000 million as the source of plutonium. As a study specially commissioned by the US Congressional Research Service concluded last year, a small nation with ambitions for nuclear weapons could satisfy them for an investment as small as \$13 million—\$26 million, by building a small gas-cooled, graphite-moderated type of reactor, capable of making about 10 kilogrammes of plutonium a year. The design data for such a 'plutonium furnace' is already freely available in the open literature, and all the materials and components with the single exception of uranium—which is widely distributed across the Earth's surface—can be bought on the open market.

The UK Government estimates that about a dozen nations already have the basic knowledge and access to fissile materials. By 1987 another 20 could have reached this position. In short, it is already too late to prevent other nations from joining the nuclear club if they are determined to do so. A far more persuasive deterrent would be to create an international climate sufficiently hostile to nuclear proliferation to persuade any would-be aspirants that they would lose much more than they could possibly gain by possession of nuclear explosives. So far this has manifestly not been the case. □



Plutonium fuel pins

USA

Feeling the draft

Legislation to control experiments with recombinant DNA is progressing slowly through Congress. Colin Norman reports

THREE months ago, when Congress first began to consider legislation to regulate recombinant DNA experiments in the United States, many scientists were hoping that a straightforward bill, establishing a uniform set of national controls, would swiftly be put together and passed without too much wrangling. Congress has not obliged, however. When the bill finally emerges, it will be a lengthy and complex document, establishing an elaborate mechanism for setting new regulations, ensuring that they are followed, and providing punishment for those caught flouting the rules. Moreover, a good deal of political manoeuvring is still in store before the bill emerges in final form from the Congressional mill.

At least the legislative machinery is beginning to pick up speed, however. Last week, key committees in both the House and the Senate passed their own versions of legislation, paving the way for consideration of the measures by the full House and Senate in the next few weeks. The House subcommittee on Health, headed by Representative Paul G. Rogers, approved a bill running to 46 pages; it will now be considered by the full Commerce Committee and possibly by the Committee on Science and Technology, before being sent to the House floor. It is unlikely that the draft approved by the health subcommittee will be substantially modified at any of those stages, however. In the Senate, the Committee on Human Resources last week approved a 56-page bill drafted by staff members of the Senate Health subcommittee, headed by Senator Edward Kennedy. It will probably be sent to the full Senate next month.

The two versions differ significantly in their approach to regulating recombinant DNA research, and thus a Senate-House conference committee will eventually be given the task of putting together a compromise bill.

Though there are substantial differences in the two bills, there are a number of important points of similarity. Both would require new safety regulations to be established, governing all recombinant DNA experiments in the United States. They also both require that facilities in which recombinant DNA experiments are con-

ducted must be licenced, that violators be subject to heavy fines, that extensive records be kept, and that federal inspectors have a right to investigate facilities and records. They also call for a study of long-term implications of genetic engineering, and they both come close to specifying that the federal regulations should take precedence over controls imposed by state or local governments. The big difference is that the Senate version would establish a Presidentially-appointed regulatory commission to draft and enforce the regulations, while the House version would leave much of the day-to-day enforcement to local biohazards committees.

The House bill, though largely written by committee staff members, was put together during three lengthy committee meetings at which about half-a-dozen Congressmen took an active interest. The chief provisions:

- The Secretary of Health, Education and Welfare (HEW) would be required to draft new regulations governing recombinant DNA research. They would prescribe physical and biological containment conditions for different categories of experiments, set out training requirements for laboratory workers and researchers, specify systems to monitor for the escape of potentially hazardous organisms, require medical surveillance of laboratory personnel, and describe reports and records which must be maintained in research facilities. The regulations would have to be published within one year after the bill is passed, and they would come into effect six months later. In the meantime, the NIH guidelines would be made applicable to all recombinant DNA experiments, no matter whether they are conducted in public or private institutions.

- A part-time advisory committee, consisting of 17 members appointed by the Secretary of HEW, would be established to assist in drafting and implementing the regulations. A majority of the members would be people who are not engaged in recombinant DNA research, and the committee would include a laboratory worker and a representative from industry.

The guts of the bill is a provision establishing a mechanism for licencing facilities housing recombinant DNA experiments. An application for a licence would have to contain a thorough description of proposed experiments, including experimental protocols, assurances that the regulations would be followed, and an analysis of the potential risks to public health or

the environment arising from each proposed experiment. The application and granting procedure would vary according to the type of experiment proposed, but in every case, local biohazards committees would play a strong rôle.

These would be committees consisting of between 9 and 15 members, including at least one laboratory worker and one local public health official. A majority of the committee members would be scientists. The biohazards committee would review all licence applications for facilities under its purview, and it would have the power to grant licences for experiments requiring low containment levels—P1 and P2 under the NIH guidelines. For research requiring P3-level containment, the licence application would have to be reviewed first by a biohazards committee, and subsequently by the Secretary of HEW and his advisory committee. The Secretary would be able to override a negative recommendation by his advisory committee, but in practice such a decision would be unlikely. A similar procedure would be required for applications involving P4-level work, but in such cases the Secretary would be able to grant a licence only if his advisory committee recommended approval.

It should be noted that, by specifying different arrangements for experiments requiring P1, P2, P3 and P4 levels of physical containment, the legislation presumes that such levels will form the basis of the final regulations.

The House bill would leave most of the inspection of facilities and monitoring to the biohazards committees, though federal inspectors would also be empowered to check to ensure that the regulations are followed. Violations could result in revoking a facility's licence, and in addition researchers could be subject to fines amounting to \$5,000 per day for each offence. Particularly serious and wilful violations could result in a \$50,000 fine and up to a year in prison. The bill also provides legal protection for workers who blow the whistle on their employers by bringing incidents of flouting the rules to public attention.

The bill approved by the Senate committee would establish a very different regulatory framework. It would set up an 11-member National Recombinant DNA Safety Regulation Commission to draft regulations, issue licences for facilities and monitor compliance with the regulations. The chairman would be a full-time official, while the other commissioners would be part time; all would be appointed by the President. A majority of the commission members would be non-

biologists.

The commission would be required to issue new regulations within 120 days after the bill is approved; they must be at least as strict as the NIH guidelines. The Senate bill also requires that all facilities housing recombinant DNA experiments must be licenced, and licence applications must specify the proposed experiments, the names of investigators and their qualifications. The Commission would be responsible for granting all licences, but initial review and approval of recombinant DNA projects would be undertaken by local biohazards committees. At least one third of the members of the local committee must be non-biologists, and at least another third would be employees of the facility applying for the licence.

The Senate bill also specifies that federal inspectors would have the right to enter facilities to inspect records and equipment. It also requires extensive record-keeping by researchers. As in the House bill, the Senate version specifies that violations could result in denial of a licence, and violators could be subject to a fine of \$10,000 per day.

Though the two bills would probably not lead to the adoption of radically different regulations, they would lead to different forms of bureaucratic control. In particular, a number of scientists have expressed concern that if a large commission, backed by a full-time staff, is established to review and approve each licence application, there could be considerable bureaucratic delay. One scientist also suggested last week that a Presidential commission to oversee recombinant DNA research would amount to 'overkill'.

One of the most controversial points in the legislation has been the question of whether federal regulations should override controls imposed by state or local governments. In fact, that was the only point which was even discussed during the Senate committee's consideration of the bill last week. The House and Senate versions are not greatly different in their requirements. The Senate bill would allow local governments to set stricter controls only if the extra safety precautions are "material and relevant" to deal with "health and environmental concerns or comparable compelling local conditions", and if they are not "arbitrary or capricious". The House bill would allow stricter local controls only if the Secretary of HEW determines that they are "necessary to protect public health or the environment".

The bills are not likely to reach the floor of either the House or the Senate much before the end of July, and it will then take several weeks for a conference committee to draft a compromise between the two versions. □

● An intensive lobbying campaign by space scientists has won a reprieve for two high priority projects, the Large Space Telescope and the Jupiter Orbiter Probe. A key Senate appropriations subcommittee last week voted funds for both projects, in spite of opposition from Senator William Proxmire, the subcommittee chairman. The vote will almost certainly ensure that the telescope will go ahead as planned, but prospects for the Jupiter project are a little less certain.

The Large Space Telescope seems assured of Congressional support because it has already been approved by the House of Representatives. Last week, the House passed a budget bill for NASA which contains funds to begin building the instrument in the 1978 fiscal year. Thus, if the full Senate follows the recommendation of Proxmire's subcommittee, which is likely, both the House and Senate versions of the NASA budget bill would contain funds for the telescope, thereby virtually guaranteeing final Congressional approval for the project.

The Jupiter Orbiter Probe is still in some trouble, however, because no funds for the project were included in the House bill. The House Appropriations Committee had specifically deleted the money from NASA's budget request, arguing that approval of two major space science projects in one year cannot be justified. The space telescope has higher priority, the committee said, and therefore the Jupiter project should be dropped. The full House followed the committee's recommendation.

It thus seems likely that the Senate version of the NASA budget bill will contain funds for the Jupiter project, the House version will not, and the matter will have to be decided by a House-Senate conference committee. NASA is hoping that it can persuade the conference committee to approve the project.

It is likely that the conference committee will in fact provide some funds for the project, but not as much as the \$20.7 million that NASA requested for 1978. In that case, NASA would at least be able to begin building the spacecraft and it would be able to avoid laying off scientists and engineers at the Jet Propulsion Laboratory, which will manage the project. The spacecraft is scheduled to be launched late in 1981 or early in 1982.

● President Carter's plan to slow down the development of a commercial liquid metal fast breeder reactor is,

like other key elements of his energy policy, being severely mauled on Capitol Hill. Carter had proposed that a demonstration breeder reactor, scheduled to be built on the Clinch River in Tennessee, should be deferred indefinitely, and he recommended that virtually all the funds for the project should be deleted from the budget for the Energy Research and Development Administration. Last week, however, the House Committee on Science and Technology voted by 19 to 11 to approve the Clinch River plant and it restored \$150 million to ERDA's budget to begin constructing the facility next year.

The vote far from guarantees that the Clinch River plant will be saved from the axe, but it does at least ensure that there will be a full-scale debate on the matter when the ERDA bill reaches the House Floor. Senator Howard Baker, who represents Tennessee, reportedly said last week that the vote has made him "optimistic for the first time" that the plant will be reprieved.

Carter's recommendation that the Clinch River plant should be cancelled was a key part of his plan to prevent the use of plutonium as a reactor fuel. He also proposed that the United States should defer indefinitely the reprocessing of spent reactor fuels to recover plutonium for recycle in light water reactors. At the same time, the Administration is trying to dissuade other countries, particularly those which do not possess nuclear weapons, from entering the so-called plutonium economy.

The House Committee on Science and Technology was persuaded, however, that other countries would not defer their plans to recycle plutonium and build breeder reactors. The committee therefore argued that the United States should not allow itself to be deprived of a technology which other industrialised countries are vigorously pursuing.

Similar statements have also been made by Senator Frank Church, who heads a key energy research subcommittee in the Senate, and by Senator Henry Jackson, who chairs the Senate Committee on Energy and Natural Resources. Their support for the breeder programme is almost certain to force a full debate on the Clinch River plant in the Senate.

Thus a protracted debate on Carter's plutonium policy seems assured in both the House and the Senate. At this stage, nobody is willing to predict the outcome.

Colin Norman

EEC

Council flat

The EEC Council of Energy Ministers and Council of Environment Ministers both met in Luxembourg last week. Patricia Kelly and Chris Sherwell report

THE two councils held last week shared a common vein. There were no concrete decisions; and a mass of texts was referred back to national experts for further discussion. Thus it was hardly surprising that one of the most useful discussions demanded no decisions in the first place. That was the debate in the energy council on nuclear energy.

The debate had a status analogous to the open discussion at the March council on energy conservation. The Energy and Research Commissioner, Dr Guido Brunner, worried about negotiations with the US and Canada, wanted the session behind closed doors, but no one agreed. Discussion centred on Community problems relating to uranium supplies, reprocessing and waste management.

More specifically, the idea of Community responsibility for safety aspects of the fuel cycle was discussed. Unsurprisingly, some concern was expressed at the idea of handing over responsibility on a matter where political control could prove so important. The question of Community participation in the high level study set up by the recent London summit was also raised, but not pursued.

On nuclear power forecasts, Britain, together with France and Germany, found the Commission's 1985 projections optimistic. Britain also criticised a suggestion, in a Community paper prepared for the meeting, for a high temperature reactor project—memories of Dragon are still fresh. There was no discussion of the vexed problem of nuclear accounting, over which a controversy, involving past uranium shipments, had blown up since the last council. The matters of nuclear costs, of public opinion and of the fast breeder could only be mentioned.

In restricted session the UK Government refused to accept what it interpreted as an enforced share-out of its North Sea oil with the rest of the Community in the event of a new world energy crisis. It was agreed that in the case of an emergency, EEC headquarters would order a 10% cut in oil consumption for two months. The Commission wanted the percentage of the cut for those countries that cannot easily find substitutes for oil to be calculated in terms of that country's oil

consumption only; in countries where substitutes are more readily found, this percentage would depend on total energy consumption including oil. Italy's Carlo Donat Cattin was anxious that the Italian dependence on oil should be safeguarded in the event of a crisis. But Britain's junior minister, Dr Dickson Mabon, said that as the Community's only major energy producer, the UK was not prepared to cut its consumption more than other countries at the dictation of the Community.

It was clear that while the UK Government would be ready to help those countries most affected by an energy crisis, it could not accept a decision taken by a qualified majority vote in the council. Britain was the only country that did not want the council to depart from unanimous voting. Ministers instead agreed that if a structural problem was affecting any member state, should the Commission order a cut, the council would undertake to meet within ten days of specific proposals from the Commission.

On the matter of the minimum safeguard price for oil there was apparently little effective discussion. But the Dutch minister, Rudi Lubbers, raised the question of long-standing Dutch proposals on energy prices generally. He argued that EEC energy prices should be brought up to oil parity to encourage conservation. But Dr Mabon commented that it was too early for common pricing. The council also disagreed about Commission proposals on how to tackle overcapacity in the Community's oil refining industry. The talks founded on the consultation system where governments would have to seek the Community's approval for new refineries to be built. On top of this, there was no discussion at the council of proposals to regulate financial support for projects exploiting alternative sources of energy.

Three coal texts were among proposals sent back for further discussion. These were the monitoring of imports from third countries, the creation of an EEC financial aid scheme to encourage the burning of coal in new power stations and a system of EEC financial support for coal stocks.

The UK Energy Secretary, Anthony Wedgwood Benn, in the chair for the last time before the new Belgian Minister for Economic Affairs, Willy Claes, takes over the council presidency, said that it had been a solid six months of work during the UK presidency. At Mr Benn's initiative, the council has agreed to hold a general debate at each energy council, on EEC

energy and on world energy.

As for the Environment Council, this seemed doomed from the start. One observer commented that 'red mud', a by-product of the titanium dioxide industry, not only polluted the marine environment but also seemed capable of polluting the atmosphere—notably between ministers. The Italian delegation made it clear that if the council failed to agree with Commission proposals to control waste from the TiO_2 and paper pulp industries—issues on which agreement has long been sought—then it would not agree to a single item on the agenda. The Italians stuck to their guns.

The Nine were split on the old question of uniform emission standards versus quality objectives as a means of control. The British argument, backed up by Ireland and Denmark, is that their industries discharge into tidal waters able to cope with large quantities of pollutants, making the Commission proposals environmentally unnecessary as well as costly to fulfil. France and Italy would like to see all Community countries impose the same controls to avoid putting industries at a competitive disadvantage. Britain maintains that what it gains on environmental cost advantages is lost on transport against more centrally placed Community countries, and was quick to point out that the dual approach to pollution control was accepted by the Nine in 1975 in a directive on the discharge of dangerous substances into the aquatic environment.

Thanks partly to the work of a working group set up during the council, some way of proceeding further may be possible, based on a distinction between reducing pollution caused by waste from an industry and reducing the quantity of the waste.

The council continued in spite of the Italian reserve. Ministers approved a decision establishing a common procedure for the exchange of information on the quality of surface fresh water in the Community. It will be formally adopted when the Italian reserve is lifted. They failed to finish discussion on EEC accession to the Helsinki Convention on protection of the Baltic. Authorisation to open negotiations is expected. Differences between the Dutch and countries through which the Rhine flows before reaching Holland meant no agreement was possible on proposals on the quality of drinking water. The proposals go back to Coreper, the committee of EEC ambassadors, for further discussion. So do proposals on toxic and dangerous waste on which the council did reach a consensus on the main aspects; a directive is expected soon. □

JET

Stalling point

RIDING on the back of a busy day in Brussels last week when the Councils of Energy and Environment Ministers both met (see opposite), the controversial issue of the EEC's Joint European Torus (JET) fusion project blew up again, as it was always destined to do. JET itself has not yet blown up, and for many that is some comfort. But the possibility nevertheless remains, and the absence of a decision on a site for the project, which is and always has been the crucial issue, now symbolically represents all the current problems of cooperation that the EEC would dearly like to avoid.

The background is familiar. Of the original sites proposed for the project, four stood out: the EEC's research establishment at Ispra in Italy; Cadarache in France; Garching in Germany; and Culham in Britain. Italy held out for Ispra until its future was more or less guaranteed through judicious deployment of the EEC's joint research programme for 1977-80. France held out for Cadarache, with CERN as an alternative, until it could do so no longer—which was effectively until it could secure a better deal to present back home. That left Garching and Culham.

The character of that 'deal' is not so familiar. But it was crucial to the failure of the 29 March meeting of the Council of Research Ministers at which the whole matter was to have been finalised. No one talks about deals in the 'open' Community, so even the most popular theories are speculative. But basically the idea seemed to be that Britain would at last have the Community project it wanted by receiving JET, that the project director would be German, and that France's proposed management structure would be used. Agreement would then have been possible on a majority vote.

But something went wrong. The small countries, already apparently miffed at the failure to agree on farm prices earlier that week, objected to the ostensible collusion. The majority shifted and the arrangement, inasmuch as it existed at all, began to fold. Details of the proposed management structure, which apparently involved the creation of a JET board of managers with a degree of detachment from the European Commission, also became an issue, making a decision on the site seem pointless.

At the same time, however, final

approval was somewhat surprisingly given in a separate vote to the joint research programme. Until then the issue was inextricably linked to the matter of the JET site; now the link seemed broken. In fact it wasn't, because there was still the all-important matter of releasing the agreed funds for the programme. This Britain has since refused to do, maintaining that it got its fingers badly burned in March when, in the Energy Council meeting on the same day as the Research Council, its concessions on the Euratom loans scheme unexpectedly failed to yield agreement on a minimum safeguard price for oil. Agreement on that issue had been expected in turn to encourage the intended agreement involving JET and the research programme.

All this left the Community with a need both to finalise details about the management of JET and, as usual, to try to reach an understanding on the site before the next Research Council. Meetings of officials proceeded accordingly. But no meeting of the council was set for before the end of June because it was only then that Britain's chairmanship, by now under suspicion, was due to give way to Belgium's. That spelled trouble, because contracts on which the JET design team at Culham were working were also due to expire at the end of June.

Although the Commission was given the authority in March to extend the project beyond June, and presumably has whatever money it needs to do so (the team's salaries are in fact paid by their own home laboratories), a decision on the future of JET before the end of June could only come in a meeting of the Council of Foreign Ministers which was due this week, on 21 June, or next week, at the heads of government meeting on 29 June. Hence all the activity last week, even though JET was not the business of the Energy Council, nor, strictly speaking, the regular business of the foreign ministers or prime ministers.

The sudden burst was typical of the way the Community conducts its business. Guido Brunner, the Energy and Research Commissioner, on the day before the Energy Council, warned that the absence of a decision before the summer recess—at the Foreign Council or heads of government meeting, in other words—would amount to an abandonment of the

project. He was responding to a telegram received from the JET design team expressing concern over the problem; he had also reportedly received a telegram from Ispra staff complaining about the hold-up in research programme funds which they thought was threatening their jobs.

Then JET came up as a spin-off from the Energy Council when at a press conference afterwards the UK minister Dr Dickson Mabon reminded people of Britain's strong desire to have JET. A parliamentary question in the House of Commons later produced the answer that, although remaining differences had recently been narrowed, if discussion was still in progress by the end of June, "we hope the Commission will again extend the contracts of employment of the design team".

On top of this came allegations in the European Parliament, which were spurned by the UK official there, that Britain's hold-up of research funds amounted to blackmail. The official had admitted publicly that two separate issues were intimately tied together as a political package. But he assured listeners that there was enough money for the research centres to continue work and pay salaries until the autumn.

Then later last week came reports that behind-the-scenes contacts were under way between London, Brussels and Bonn involving third party mediation, perhaps from the next country to hold the chair, Belgium, in an effort to secure a solution before the end of the month.

That must be the fervent hope of all those who want to see the project survive. Yet it remains that the lack of trust Britain has felt over JET since the March débâcle is now almost fully reciprocated by other member states on this as well as other issues. Both sides now want impossible guarantees of a *quid pro quo*, and refuse to move until the other does.

Meanwhile a date for the next Research Council had at the end of last week still to be set. If nothing is achieved by the end of the month, then what is called an "orderly winding-down" of the project will begin. In other words, like the other European project, Dragon, which died last year in such ignominious fashion, JET too could be cancelled for lack of interest.

Chris Sherwell

COMECON

● The recent concern in the West about the dangers of nuclear power evoked a uniform and perhaps predictable response from the Comecon media; such fears, it is alleged, are the result of political scare-mongering and/or capitalist negligence. The nuclear power plants that are designed and built in the Comecon bloc on the basis of Soviet R&D, it is claimed, constitute no hazard to health or the environment.

This confidence, although it may be excellent propaganda, hardly constitutes a sound basis for planning an energy policy which is heavily committed to a change-over to nuclear generation as rapidly as construction and logistical problems permit. (It is estimated that by 1980, some 10% of the generating capacity of the whole Comecon bloc, including Cuba and Mongolia, will be nuclear.)

The risk of accidental pollution, however, is appreciated by the planners. When the Institute of Radioecology and Nuclear Technology was founded two years ago at Kosice, Czechoslovakia, systematic research was begun, not only into the prevention of accidental escapes of radioactivity, but also into the ecology of living organisms in an environment polluted by radioactive waste. According to Milan Zaduba, the Director of the Institute, this is the only research institute in the world making such a study. This unique position, however, will not be maintained for long, since future plans for the institute envisage the gradual transformation of its activity into a programme of multinational cooperation with institutes in the Soviet Union, Poland, and the GDR.

● Bulgaria, as one of the less-developed members of Comecon, has a science and technology programme which is closely coordinated to that of the Soviet Union. At present, there are more than 30 agreements between the two countries involving 17 Bulgarian and 48 Soviet ministries and 20 bilateral working groups. To date, the flow of information has been largely in one direction, the Soviet Union having supplied Bulgaria with two-thirds of her machinery, equipment and complete plants, and with over 6,500 complete sets of technical and scientific documentation.

The flow of information, however, is now becoming somewhat more balanced. Uses are being found for Bulgarian technical documentation and a number of Bulgarian innovations are being introduced into

various branches of Soviet industry. Recently, a joint Soviet-Bulgarian Institute, Interprograma, was founded in Sofia, to develop software for automated control systems for production and management. The name of the institute is reminiscent of numerous Comecon projects and co-



ordinating centres, such as Interkosmos and Interatominstrument, and suggests that the new institute may possibly be expanded, in the future, into a similar multinational body.

The same possibility is also suggested by the recent agreement setting up Intervodochistka, the Comecon international R&D corporation dealing with problems of water conservancy and purification. Under this agreement, Bulgaria is charged with producing the various digital automated units which the corporation will need. From the communique issued by the 80th session of the Comecon Executive Committee in April, it would appear that Bulgaria's responsibility will be extended to providing similar equipment for other branches of the economy.

● Poland's inland waterways have been a matter for concern over a number of years, but it was not until January 1976 that a government decision finally settled the long-standing debate as to whether the waterways were worth modernising and developing at all. By this time, a number of weirs and locks had become urgently in need of repair, but in some cases, the conflicting demands of other sectors (notably the Ministry of Agriculture) have meant that repair work has had to be abandoned. Moreover, during the years of neglect, the waterways had become a convenient means of dumping industrial effluent and sewage, and now only 53% of effluent is treated at all,

the rest being allowed to discharge into rivers and lakes. Some 28% of the total length of the rivers is said to be "beyond economic utilisation".

At present, water consumption in Poland is 13,000 million cubic metres per annum, and is expected to rise to 18,000 million cubic metres in the next few years. While the civil engineers are struggling to produce a rational scheme for the renovation of river installations to cope with modern traffic, plans are also being discussed for the reduction of pollution. Suggested remedies include the use of more easily soluble detergents and pesticides, the recycling of agricultural effluent, and the development of water-saving technologies. One factory, the new Gorazdze cement works in Opole province, however, seems unlikely to add to the pollution problem. Although this plant is sited near the Odra, it needs the river as a means of transport only; the raw materials are prepared by a dry pulverisation process. "The advantages are obvious," commented *Dziennik Ludowy*. "Where there is no water, there is no effluent."

● According to Yu. Maksarev, the Chairman of the State Committee of the Soviet Union on Inventions and Discoveries, one in three of all patent applications in the world is made in the socialist countries. To cope with this vast flow of invention, the Comecon countries are at present engaged on working out a system to improve the national patent information systems of the member countries and also, by means of "division of labour, cooperation, and the centralisation of individual information processes", to increase the volume of information exchanged between member countries.

According to Maksarev, invention in the Comecon member countries is based on a study of world patent information. This study has not, it would seem, had any marked effect on the type of information patented by the Soviet Union, which, with some 100,000 'author's certificates' published in the last three years, is responsible for the majority of Comecon inventiveness. A close study of these 'certificates' reveals that in a significant number of cases, the novelty would be insufficient for a patent to be granted in any Western country, being at best the type of innovation which would be registered as a 'utility', and at worst simply minor modification which, in the West, would be incorporated as an unregistered factory improvement.

Vera Rich

WINDSCALE

Down to business

The Windscale inquiry opened in Whitehaven, Cumbria, last week. Chris Sherwell reports

NO ONE thinks a decision on Windscale is going to be easy; the case is too finely balanced. But practically everyone involved in the inquiry, on the application from British Nuclear Fuels Ltd (BNFL) to develop land there for an oxide fuel reprocessing plant, hopes that the decision will go their way. Once Mr Justice Parker has made his recommendations, probably by the autumn, the responsibility will lie with the Secretary of State for the Environment. Assuming the Labour government survives until then, that will be Mr Peter Shore.

Opening statements from BNFL and from the various opponents of the project were heard last week. Cross-examination of some 18 witnesses to be brought by BNFL also began. The first was Mr Con Allday, the managing director, and he was still on the stand on Friday. Before he appeared the inquiry already looked like broadening the spread of issues it will cover when Mr Justice Parker produced over a dozen points on which he said he and his two assessors wanted early clarification from BNFL.

The points drew attention to figures showing that plutonium recovered through reprocessing would be enough to charge more fast breeder reactors than the country could possibly need. Mr Justice Parker said they wanted to know if one objective was to keep the breeder option open. They also wanted to know how much plutonium was required to fuel a breeder and how much had been recovered and would be recovered from spent Magnox fuel.

The implication was that existing facilities at Windscale for reprocessing oxide fuel—using the 'head-end' plant which is presently inoperative—might cope with domestic reprocessing demands and take some overseas business, and with other work also satisfy Britain's plutonium requirements. In short, was a new plant necessary?

This all fitted with what Mr Justice Parker had said at the start of the whole proceedings about three basic questions needing answers. These were: should spent oxide fuel be reprocessed in Britain at all? If yes, should it be reprocessed at Windscale? If yes, should the plant be larger than that required for domestic purposes in order to reprocess fuel from abroad?

In the context of these questions it

was hardly surprising that the precise terms of the Japanese contract that BNFL has been trying over many months to negotiate also became important soon after Mr Allday took the stand. He was reluctant to furnish the document, yet plainly, without the terms of the proposed contract, no detailed assessment could be made of the financial and economic factors which, for the third question at least, BNFL themselves must regard as relevant. Sight of the terms was subsequently allowed.

Under cross-examination Mr Allday admitted it would be cheaper to use refurbished or rebuilt facilities that existed, but said this would not be enough. He also endeavoured to explain why existing facilities to reprocess spent Magnox fuel from Britain's first generation reactors had failed to operate at full design capacity. He attributed this at least partly to the difficulty of handling spent fuel elements that were in a poor condition because they had stayed in reactors too long. Also broached was the question of what work was being done on dealing with fuel if it should not be reprocessed.

Earlier in the week the inquiry had opened amid a burst of publicity generated by the presence in Whitehaven of nuclear experts, company men, government officials, lawyers, environmentalists, demonstrators and journalists. Many detected a note of resentment among the local people who have to live with Windscale. But the resentment was less at the plant, expansion of which means more jobs, than at the foreign invasion. Non-BNFL voices supporting the project because of the benefits it would bring to the area came from Cumbria County Council and Copeland Borough Council. But another authority, the Isle of Man, is among the objectors, not all of whom agree on the basis from which their arguments can best be launched.

Before the inquiry opened BNFL confirmed that a laboratory at Windscale had been closed following an accident six weeks earlier in which a pressurised glove-box had blown back and released plutonium. During the week, as part of a new policy of directly releasing details of all accidents no matter how trivial, BNFL published a list of incidents that had occurred over the previous two months. These details are usually passed on to the Nuclear Installations Inspectorate and to the Secretary of State for Energy.

The inquiry continued this week with more evidence from Mr Allday. Other BNFL witnesses were also to appear. □

URANIUM

Hot topic

A TEAM of experts from Australia is thought to be in Europe to discuss possible sales of uranium for EEC nuclear power stations. They were expected to visit Brussels to discuss marketing arrangements and review Euratom security controls. The Australian Prime Minister, Mr Malcolm Fraser, visited Brussels last week for talks with the European Commission. He told reporters afterwards that his government had not yet decided on its export policy for uranium. An announcement is expected within weeks now that the second report of the Fox Commission has been published.

In spite of speculation to the contrary, Mr Fraser said that there was no question of a trade-off between possible exports of Australian uranium and greater access to EEC markets for Australian agricultural produce. Overall commercial relations between Australia and the EEC can, however, be expected to influence the matter, and Mr Fraser has attacked the EEC's Common Agricultural Policy.

Mr Fraser would not answer questions about Australia's alleged membership of a world-wide uranium cartel, into which further probings were made in the United States last week when a bundle of internal memoranda of the Gulf Oil Corporation was released by a Congressional subcommittee. They seriously undermine Gulf's contention that it was an unwilling partner in the cartel, and contradict its assertion that the operation of the cartel had no effect on domestic uranium prices in the United States. The memoranda, which were supplemented by a three-and-a-half hour grilling of Gulf's chairman, Jerry McAfee, could pose serious problems for Gulf's defence against possible anti-trust suits.

McAfee persistently claimed at the hearing that Gulf's Canadian mining subsidiary was pushed into the cartel by the Canadian government. He did not give details, however, and some of the internal memoranda depict the company as a willing partner. One memorandum even discussed how the cartel could eliminate competition from other uranium companies. The cartel was formed in 1972, and operated until 1974, a period when uranium prices increased sharply. McAfee and other Gulf officials admitted in their testimony that the increasing world price of uranium had, to a limited extent, also driven up domestic prices in the United States.

The second annual meeting of the Uranium Institute was meanwhile due to take place in London this week. The institute, established in June 1975, exists "to promote discussion between uranium producers and consumers", particularly on uranium supply and demand. It has 36 members, including 21 producers and 9 consumers. There are no US members. The institute's secretary general, Mr Terry Price, was among those called to give evidence before a US judge at the US embassy in London last week in connection with a case brought by Westinghouse Electric Corporation concerning the cartel.

IN BRIEF

Petition to Poles

Three Nobel prize-winners—George Wald (medicine), William Lipscomb (chemistry) and Kenneth Arrow (economics)—and some twelve other well-known American scientists are among the 139 signatories of a petition to the Polish government asking for the immediate release of the imprisoned members of the Workers' Defence Committee "whose only crime consists of defending the basic human rights of others".

Meanwhile it is reported from Moscow that the physicist Professor Mark Azbel has, exactly six years after his first application, been granted permission to emigrate to Israel. Since 1974, when Professor Alexander Voronel went to Israel, Azbel has been chief organiser of the illicit Sunday Seminars for refusenik scientists.

UK joins in on test ban

The Soviet Union and United States began talks in Washington last week on how to achieve a comprehensive nuclear test ban. The Russians are reported to be keen to come to some agreement on a test ban and President Carter is said to have made it a priority in his programme of arms control.

As the result of a Russian request Britain will join the two super powers, starting 13 July, in drawing up a comprehensive nuclear test ban treaty. The three countries have collaborated before on nuclear arms control, in 1963 on the partial test ban treaty and in 1968 on the non-proliferation treaty. It is thought likely that Britain will go along with a comprehensive treaty provided that it includes nuclear explosions for peaceful as well as military purposes.

Neutron source

Rutherford Laboratory has been given the go-ahead to build a spallation neutron source. The announcement, during the official opening of the laboratory's laser facility (see page 666), greatly eases the transition of the Rutherford into a new era.

Nimrod, the laboratory's 7 GeV proton accelerator, is due to close in 1978, and although the new laser facility goes some way towards using the laboratory's expertise, there had been fears that in the absence of further projects there would have to be redundancies. The spallation neutron source, however, in which 800 MeV protons pulsed at 50 cycles per second will be fired at a heavy element source to break up nuclei and yield fast neutrons, will use much of the existing human and hardware investment in Nimrod.

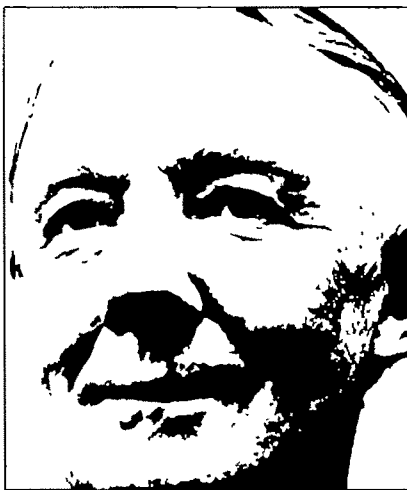
I recently attended the 1977 Sponsored Film Festival, organised by the British Industrial and Scientific Film Association and held in London during ten days in May. During that period 188 films and video tapes were shown, and numbers of prizes were awarded by distinguished panels of judges. The films were divided into ten categories—education, training, safety, technical, scientific and research and so on. I restricted my attendance to "Ecology and the Environment".

This was an interesting, and, to me, a new experience. Although in my youth I was an enthusiastic film goer, witnessing the change from the silent film to the 'talkie', and the peak of the industry's prosperity, I have not been inside a commercial cinema for several years. I occasionally watch old feature films on television, and have often seen, and used, short scientific films at lectures and conferences. I have even been concerned with making some such films. But I have never before sat from 9.30 am to after 5 pm watching a continuous series of moving pictures.

I found that the organisers of the festival had given our theme a wide interpretation, for in addition to seeing productions about energy and pollution, nature reserves and the protection of wildlife, we were also treated to propaganda films trying to persuade us to visit Guernsey, the Lake District or the historic castles of England and Wales.

In one way or another, the oil industry was prominent. The government's Central Office of Information screened "Clean and Pleasant Land" showing the horrors of oil pollution on the sea and the beach, and how

Britain was coping with the problems. Two potential polluters, British Petroleum and Shell UK brightened their tarnished images with an admirable sermon on energy saving and a pic-

Ecology on film**KENNETH MELLANBY**

ture of the, as yet, unspoiled Peak District National Park.

Most of the rest of the programme was more truly environmental and ecological. The Royal Society for the Protection of Birds, Britain's largest voluntary conservation organisation, showed two films made by their own unit. In the first, David Bellamy, one of our leading telly-dons, described the dangers to our small areas of surviving heathland. In the second, Ian Prestt, the Society's Director, spoke persuasively about its work. These combined propaganda with education, and included good and unusual natural history photography.

However, as I watched, I wondered if some films might not, by their very excellence, defeat their own objectives. For instance "The Petersfinger Cuckoos" enabled the cinema audience, and those who watched it on television, to observe the most intimate details of the private life of this elusive and parasitic bird. It all looked so easy, yet we know that "the few seconds of the secretive cuckoo laying its egg took more than 1,000 hours of waiting and watching". I have sometimes been worried lest inexperienced town-dwellers who have watched such programmes may be discouraged when they visit the country and see comparatively little wildlife, even on a nature reserve with the help of a skilled naturalist.

Fortunately, this does not seem to be the case. The sound of the voice of a real, wild, live cuckoo seems to be appreciated all the more. When, at our home in Huntingdonshire, we open our rural and unkempt English garden to the public, to raise money for various worthy conservationist causes, we set out a simple nature trail demonstrating wild flowers ('weeds' to the conventional gardener), birds' nests and even newly-dropped fox dung. We find that most of our visitors, and all of their children, appear to be enthralled. It seems that the most sophisticated nature films are treated as something different from real life, in the same way that violence in Westerns and crime programmes seems unrelated to that experienced in our streets and after football matches. Nature films are undoubtedly useful, but they are no substitute for the real thing.

correspondence

Unemployed scientists

SIR,—Your article (21 April, page 668) describing the plight of Soviet scientists who have been refused employment and have tried to carry on their work in isolation rang a familiar bell. But, although the world receives news of these unhappy people in the Socialist countries, no mention is made of the plight of hundreds of unemployed biologists, chemists, physicists, and mathematicians in the Free World.

Any science department in Canada which ventures to advertise a vacancy gets snowed under by anywhere from 75–400 applicants most of whom are underemployed and receive no professional recognition from their colleagues. Despite this, universities go on pretending that no such problem exists, and have not taken steps to expand their faculty to alleviate it. The unemployed scientist has no faculty union, cannot apply for research grants, cannot receive travel grants, and must work in isolation. He is barred from receiving unemployment assistance unless willing to seek any type of work, thereby relegating his professional career, for which he and his parents have paid dearly, to the status of a part-time hobby.

Every government adopts certain policies for eliminating its unwanted and superfluous citizens. The methods of the Socialist and Free-World countries differ only in superficial aspects. A doctoral student whom I taught at the University of Calgary sought suitable employment for five and a half years from 1968–73, before taking his life, leaving a widow and three small children. Can we truly say that such people have suffered any less than the 'refusniks' of the Soviet Union?

Yours faithfully,

JAMES J. KLEIN

University of Regina,
Saskatchewan, Canada

Let n be the psammity

SIR,—What is the quantity of which the mole is the SI unit? It is 'amount of substance' (in French *quantité de matière*). However, Newton's definition of mass was as quantity of matter (in Latin *quantitas materiae*), and the word 'substance' carries overtones of theology and mediaeval philosophy. It is only necessary to recall the snickers

among scientists when 'amount of substance' was introduced, or the bewilderment of students who try to make sense of the expression, to realise that 'amount of substance' does not describe what it names, and that English needs a simple word for this quantity, as German has *Stoffmenge*.

Common speech does not have a word that can readily be extended to cover the concept, but several classical roots might yield a suitable word. Although grains of sand had been a Greek metaphor for uncountability, Archimedes in his *Sand-Reckoner* (*Psammités* in Greek) calculated the number of grains of sand that would fill the known universe. To replace 'amount of substance,' I propose 'psammity', evoking Archimedes' calculation and treated as if there were a Latin *psammitia* from which it derives, with combining form 'psammito-' and adjective 'psammitic'.

How would these be used? It is usual to write, "Let m be the mass . . .", and only later need it be revealed that the unit will be the kilogramme. On the other hand, at present we write, "Let n be the number of moles . . .". If instead we write, "Let n be the psammity . . .", the dimension of n is clear, but the unit, whether mole, micromole, or pound-mole, need only be specified when numerical values are given. Again, when the density of a gas is expressed in moles per unit volume we must at present speak of molar density, but this would become psammitic density. Other uses of psammity follow by simple analogy with mass and length, where common usage already distinguishes the quantity from the unit.

Yours faithfully,

GEORGE S. KELL

National Research Council,
Ottawa, Canada

Microprocessor myths

SIR,—I fear that some of the 'myths' mentioned in Basil Zacharov's article on microprocessors (28 April, page 760) were actually fluffy and insubstantial.

It is true that microcomputers cost more than microprocessor chips; more, even, than naked minis. But what is of genuine importance is this. One can now buy—and pay for—only as little computing power as one actually needs, right down to a four function calculator costing \$7. No longer must one

spend the \$7 every half hour in connection charges, and waste time keeping up on the latest system releases, just to be able to calculate small profundities on demand.

Microcomputer users may also spend inordinate amounts of time writing software, but after all, anyone making many small-scale runs, rather than engaging in massive, production number-crunching, must write a seemingly inordinate quantity of software (or spend almost as much time learning to use canned software). The microcomputer also has one overwhelming advantage with respect to the software investment: ownership and control. That control provides a modicum of assurance that one's heavy investment in software will not suddenly be destroyed or devalued at the whim of an outside party. The real question is: how can anyone afford not to use a computer small enough to own, whenever possible?

It is also true that microcomputers are not fast, complicated, and sophisticated like minis and mainframes, but the power of a pocket calculator is sufficient for a large class of tasks. A microcomputer system costing a mere \$1,700 is likely to be severely limited by the user's communication speed.

None of this is new. After all, that 'complex internal organisation' which allows a big mainframe to perform 'many concurrent activities' is customarily installed precisely so that the mainframe can simulate a hundred or a thousand small computers running at once, each serving a different user. Today, we can sometimes spare ourselves that nonsense, and simply purchase the hundred computers.

Small computing tasks have tended to be served inconveniently, expensively, and sometimes even with grudging arrogance, by traditional computer facilities. Microcomputers and LSI technology complete the low end of the continuum of computer powers. It should, therefore, not be hard to understand the microprocessor phenomenon. After all, it is practically one and the same with the calculator revolution. Even diehard advocates of mainframing everything have been known to use calculators.

Yours faithfully,

PAUL SCHICK

University of Wisconsin-Madison,
USA

news and views

New laser facility at Rutherford

from T. P. Hughes

THE Science Research Council's Central Laser Facility at the Rutherford Laboratory was officially inaugurated on 20 June. The occasion marks the successful completion and commissioning of the most powerful pulsed laser in the United Kingdom, whose output is available to University research workers for a variety of experiments.

The original proposal for a central facility came from several University groups investigating aspects of laser-plasma interactions. The experimental groups had previously built their own high-power lasers, but needed even higher powers to extend their work to higher plasma temperatures and densities and laser irradiances. They were acutely aware not only of the high cost of large lasers, but also of the difficulties of maintaining a reliable output from home-made systems, whose servicing occupied a disproportionate amount of time at the expense of the interaction experiments themselves. After extensive discussions, it was decided to ask the SRC to provide and operate a large neodymium laser, and to make funds available to update it and, in due course, to replace it with another laser as techniques develop.

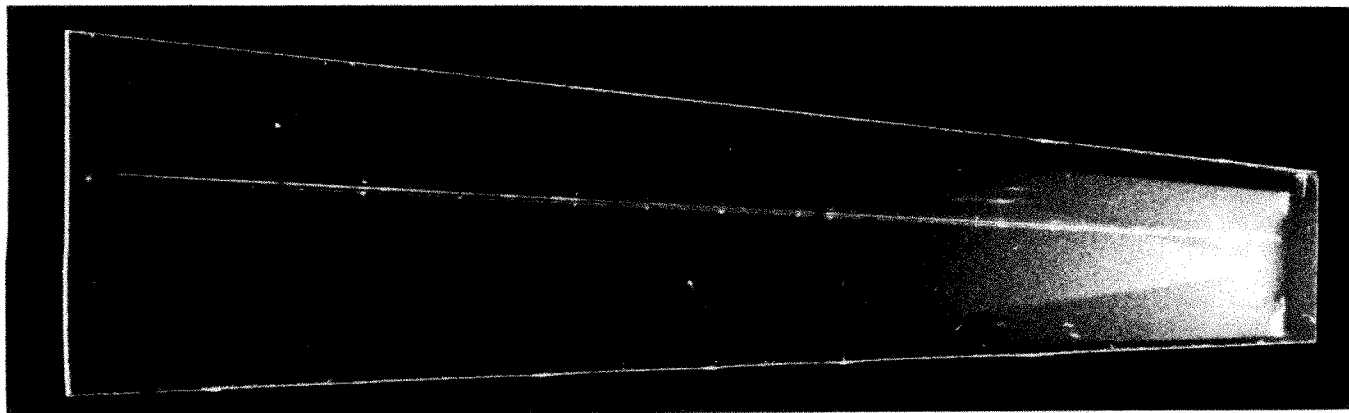
The scientific objectives of the Laser Facility are to create and study dense plasmas generated by the laser compression of matter; to study inter-

actions of intense laser radiation with matter; and to develop new and more efficient high-power lasers for these and other purposes. The creation of very dense plasmas by ablative compression of small isolated targets is currently being investigated in several laboratories throughout the world as a potential route to controlled nuclear fusion, but it has a much wider scientific significance. It is now possible to produce plasmas in the laboratory with temperatures and densities similar to those which are believed to occur in stellar interiors. Information about spectral line shapes and intensities, about radiation and other energy transport processes, and about the equation of state in these plasmas will be of great value to astrophysicists. Several other experiments using the laser facility were also envisaged in the original proposals, including the development of VUV (vacuum ultraviolet) and X-ray lasers, studies of gas breakdown and experiments in quantum electrodynamics.

Experimental programme

At a meeting of prospective users from the Universities and other laboratories in December 1975 the basis of the initial experimental programme was agreed. Detailed proposals were prepared for single-beam experiments to begin when the "driver"

stage of the neodymium laser was ready, with an output of up to 10 J in a 100 ps pulse, and for two-beam experiments using the full laser system to compress glass microballoons. During the following 12 months groups of scientists, each drawn from several universities and other laboratories, collaborated with the staff of the newly-formed Laser Division of the Rutherford Laboratory to design and construct a large target chamber and a wide variety of experimental equipment. In the meantime, the laser was being installed and tested. The first single-beam experiments began in December 1976 and continued until the end of March 1977, with several pauses to permit further work on the laser. During this period many plasma diagnostic techniques were tested and some valuable X-ray and VUV spectroscopic data were recorded, including information relevant to the interpretation of solar flare spectra. Preliminary observations of the time required for the laser pulse to burn through thin foil targets, prepared at the Rutherford Laboratory, were compared with computer calculations to obtain information about the thermal conductivity of the plasma layer. The two-beam laser system was completed in April 1977; the first shot at a microballoon target produced compression, as recorded with an



The illustration shows a space-resolved X-ray spectrogram from laser-compressed neon-filled glass microballoon.

X-ray pinhole camera. In this experiment the glass microballoon, 85 μm in diameter and with walls 0.5 μm thick, received only a tenth of the full rated power of the laser, focused with two $f/1$ lenses. Further experiments using microballoons selected, mounted and filled with gases such as neon and argon at the Rutherford Laboratory are now in progress (see illustration).

The laser pulse is formed initially by switching out a single spike from the mode-locked output of a neodymium-YAG oscillator. Its duration may be varied between 30 and 300 ps. A series of neodymium glass rod amplifiers of increasing diameter, interspersed with Faraday isolators and spatial filters, produces an output pulse from the driver stage with an energy of up to 10 J in a single beam of 76 mm diameter. The beam is then divided into two parts, each of which passes through a further rod amplifier into a 10 cm diameter disk amplifier to give a rated output of 40 J, corresponding to a total output of 0.8 TW in a 100 ps pulse. For stable operation and to prevent damage from dust particles, the laser is housed in an air-conditioned clean room: the beams emerge through ports in a partition into a separate target area. Here beam steering reflectors direct the laser pulses into the main target chamber. This chamber, which is evacuated, has more than 40 ports for windows and for diagnostic equipment. Within it, attached directly to the laboratory floor and isolated from the vacuum envelope by bellows, is a massive optical table on which are mounted the target holder and the two large aspheric focusing lenses. Focusing adjustments are made with the aid of a continuous wave Nd-YAG laser and a TV telemicroscope: the peak irradiance in the focal spot due to a single beam is in the region of 10^{15} – 10^{16} W cm^{-2} . The laser is controlled from another room which also houses recording oscilloscopes and a small computer used to monitor the laser and to acquire and process experimental data.

More than 30 University research workers from eight Universities are at present participating directly in the experimental programme. Theoretical and computational work on related topics, including notably the hydrodynamics of the compression of spherical targets, is also in progress at five Universities. For many of those involved in the experiments, accustomed to working in comparative isolation, collaborative research is a new and stimulating experience, which imposes its own discipline on their

activities in exchange for access to the best available research equipment, and for the rapid progress which is possible when new results and ideas are immediately and freely shared. The Rutherford Laboratory has, of course, been running collaborative research programmes, notably in high energy physics, for many years, and provides excellent facilities for visiting scientists.

The present programme of research will continue well into 1978. More sophisticated focusing optics, providing more uniform illumination of spherical targets, will be supplied shortly. New experiments on X-ray and VUV lasers are currently being planned. The rate of progress is limited by the availability of laser shots, and this situation is likely to persist into the foreseeable future: the Laser Facility is already as heavily loaded as staffing permits. Nevertheless, proposals for appropriate new experiments will always be welcomed. $\square$

Neutron scattering and ribosomes

from Richard Brimacombe

PHYSICAL chemistry has had a difficult time in coming to grips with the ribosome; in the past, all too many painstaking studies have led only to rather unsatisfactory conclusions, usually involving unspecifiable conformational changes or else bemoaning the complexity of intra-ribosomal interactions. This is however by no means the case with neutron scattering measurements, which are beginning to give fairly precise estimates of useful intra-ribosomal distances, the most recent contribution being a paper by P. B. Moore, J. A. Langer, B. P. Schoenborn and D. M. Engelman (*J. molec. Biol.* **112**, 199; 1977), entitled 'Triangulation of proteins in the 30S ribosomal subunit of *E. coli*'.

Neutron scattering has already been applied to the *Escherichia coli* ribosome to measure the distance between the centres of mass of protein and RNA within the ribosomal subunits, and here it appears that in the 30S subunit, centres of mass are more or less coincident (Moore *et al.* *J. molec. Biol.* **91**, 101; 1975). On the other hand, in the 50S subunit they are separated by a significant distance, indicating that the large subunit has a protein-rich and RNA-rich region, although there is some disagreement as to the magnitude of the displacement (Moore *et al.* *Proc. natn. Acad. Sci. U.S.A.* **71**, 172; 1974; Serdyuk & Grenader *FEBS Lett.* **59**, 133; 1975;

Stuhrmann *et al.* *Proc. natn. Acad. Sci. U.S.A.* **73**, 2379; 1976).

Similar neutron scattering studies are currently being made in a number of biochemical systems (for investigating chromatin and virus structure, for example) but the advantage of using neutron scattering to investigate the *E. coli* ribosome is that active ribosomal subunits can be re-assembled from their constituent RNA and proteins, which enables a reconstituted particle to be prepared in which selected pairs of ribosomal proteins are deuterated. The neutron technique not only allows the distance between the centres of mass of the two deuterated proteins to be measured, but also gives some information about the shapes of the proteins concerned. In the most recent paper by Moore *et al.* (*op. cit.*) six such inter-protein distances are described, which, when added to those previously published by the same group (Engelman *et al.* *Proc. natn. Acad. Sci. U.S.A.* **72**, 3888; 1975), gives a total of nine inter-protein distances within the 30S subunit. These are between proteins S2–S5, S3–S4, S3–S5, S3–S7, S3–S8, S4–S7, S4–S8, S5–S6, S5–S8, and the authors are able to use their data to construct a map of the relative locations of the centres of mass of S3, S4, S5, S7 and S8, in which only two distances need still to be measured in order to determine the relative placings of all five of these proteins in three dimensions.

Moore *et al.* conclude that most if not all of the proteins under study have significantly elongated conformations, with protein S7 being particularly oddly shaped. This finding is in full agreement with a number of recent studies, notably small-angle X-ray scattering of isolated proteins (for example Osterberg *et al.* *FEBS Lett.* **73**, 25; 1977), and the mapping of antigenic determinants on the ribosome surface by immune electron microscopy (reviewed by Stöffler & Wittmann in *Protein Synthesis* (eds Weissbach & Pestka) 117–202 (Academic Press, New York, 1977)), which also indicate that many ribosomal proteins are highly extended.

Similar measurements by neutron scattering are also currently being made on the 50S subunit; one inter-protein distance (between L7/L12 and L10) has already been published (Hoppe *et al.* in *Brookhaven Symp.* No. 27, IV 38; 1975), and measurement of several others is in progress. It is clear that the neutron technique has a useful future in ribosome research, and as measurements continue to be made the method will (as Moore *et al.* point out) reveal its own degree of

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self-consistency; within the three-dimensional lattice, each new distance serves as a quantitative check on some of those already measured.

The elongated nature of the proteins makes it rather difficult to compare the neutron results in any detail with other data, such as protein-protein cross-linking results, or the immune electron microscopy technique mentioned above. There is however another physical technique which gives data very similar to those obtained by neutron scattering, and this is the measurement of energy transfer between fluorescent dyes (Huang *et al. J. molec. Biol.* **97**, 443; 1975). Here the ribosome is re-assembled with two specific proteins labelled with two different fluorescent dyes, and the energy exchange between them can be measured in the reconstituted particle. Making certain assumptions, this gives an estimate of the relative distance between the centres of mass of the proteins concerned (although without giving any information on shape), but unfortunately the set of protein pairs which have been determined by this latter method does not overlap sufficiently with the set measured by neutron scattering for a direct comparison to be made. It is however obviously only a matter of time before the two sets of data do begin to overlap, and that should be something well worth waiting for. □

Surface antigens specified by the *T/t* locus

from P. L. Stern

THE idea that molecules displayed on the cell surface may allow differential recognition of interacting cells and therefore be a key factor in morphogenesis is an extremely attractive one. An important corollary to this view is that at least some genes which play an essential part in development would be expected to specify components of the cell surface. So recent observations suggesting that early embryonic cell surface antigens are specified by alleles at the *T/t* locus in the mouse have aroused a great deal of interest. Mutants in this locus affect embryonic development, sperm production and function and genetic recombination (see review by Bennett *Cell* **6**, 441; 1975). Many of these *t* mutants are embryonic lethals when homozygous and

seem to affect development at very specific stages.

In 1970 Gluecksohn-Waelsch and Erickson put forward the hypothesis (*Current Topics Devl Biol.* **5**, 281) that *T/t* products may be expressed on the cell surface and that the defects in the mutant embryos were due to malfunctions in cell-cell recognition and morphogenetic movements. Two lines of evidence from serological studies have suggested that the products of the *T/t* locus are indeed expressed at the cell surface although it has not yet proved possible to identify unequivocally any surface component as a product of any *t* allele.

One of the first candidates for a *t* locus gene product was the component bearing the F9 antigen. F9 antigen(s) is detected by an antiserum prepared by multiple immunisations of syngeneic mice with irradiated F9 embryonal carcinoma cells (Artzt *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 2988; 1973). F9 antigen is not expressed by any adult tissues except sperm and testes germ line derivatives but is expressed in normal embryos. The fertilised egg has no detectable F9 antigen expression but expression increases during cleavage up to the morula stage and can be detected until day 10 of embryonic development (Jacob *Immun. Rev.* **33**, 5; 1977).

This antigen, like the *T/t* locus effects, is expressed in embryonic cells and sperm. It therefore became a candidate for the product of an early acting wild-type allele at this locus, namely *t^{w32}* (*t¹²*). If the *t^{w32}* mutation were affecting the expression of F9 antigen, twice as many sperm from heterozygous *t^{w32}/+t^{w32}* animals as for wild type *+t^{w32}/+t^{w32}* should be required to remove a given anti-F9 activity, provided that sperm cells express each allele carried by the animal. This result was obtained by Artzt *et al. (Proc. natn. Acad. Sci. U.S.A.* **71**, 811; 1974) using quantitative absorption of anti-F9 serum; *t^{w1}* and *T* heterozygote sperm exhibited wild-type absorptive capacity. The interpretation that F9 antigen is specified by a wild-type allele at the *t^{w32}* locus is, however, made difficult by the fact that some recessive *t* lethals significantly suppress crossing over for a considerable length of chromosome 17. Hammerberg and Klein (*Nature* **253**, 137; 1975) have shown by typing H-2-associated antigens of mice carrying recessive *t* alleles, that particular *t* factors (within the same complementation group) represent a certain combination of genes in chromosome 17, a *t* haplotype, which has remained genetically interlocked in mouse populations for many generations. In consequence the differential absorptive capacity of the heterozygous sperm from wild type could reflect the activity of genes anywhere within the region of crossover suppression.

If anti-F9 serum detects the *+t^{w32}* gene product then this gene product

might be altered or absent from *t^{w32}/t^{w32}* embryos although not from other *t^{*}/t^{*}* embryos. Kemler *et al. (Proc. natn. Acad. Sci. U.S.A.* **73**, 4080; 1976) examined the progeny of crosses producing a proportion of embryos homozygous for *t^{w32}*, *t^{w5}*, *t^{w18}* and *T*. In crosses producing *t^{w18}* or *T* homozygotes, anti-F9 serum labelled nearly all the embryos similarly to normal morulae. Among the progeny of crosses producing *t^{w32}/t^{w32}* embryos, a significant proportion were not labelled. Further, the embryos lacking F9 were blocked in their development at the morula stage at which *t^{w32}* arrests development. However when the progeny of a balanced lethal cross producing *t^{w5}* homozygotes were examined, a significant proportion also failed to label with anti-F9 serum. *t^{w5}* is a lethal acting at 6 days of development and belongs to a different complementation group from *t^{w32}*. As pointed out by Kemler *et al.*, since F9 is not expressed by two different *t* haplotypes it cannot at this time be considered the product of any particular wild-type allele of *t*. However it is clear that F9 expression is affected by a region belonging to or closely linked to the *T/t* locus.

The other major approach to serological aspects of the *T* locus has been the production of antisera against *+t^{*}* sperm and absorption with wild-type sperm to remove sperm autoantibodies. *T/t* locus mutations specify serologically detectable antigens on sperm, codominantly expressed, they are not present on other adult cells so far tested (Artzt & Bennett *Nature* **256**, 545; 1975; Yanagisawa *et al. Immunogenetics* **1**, 57; 1974). Serological analysis is difficult because the antisera are generally weak in cytotoxic reactions with sperm. Cross absorptions of anti-*t^{*}* sera with different *t* haplotype sperm have shown that these antisera detect multiple specificities, some of which may be unique for a given *t* haplotype. However it cannot be excluded that anti-*t^{*}* sera detect, in addition to a genuine *t^{*}* antigen, some other unknown antigen specified by a gene linked to *T/t*.

The experiments of Kemler *et al.* have shown that the *t* antigens defined on sperm are indeed expressed on embryos carrying the appropriate *t* haplotype. A sizeable fraction of embryos from crosses yielding *+t^{*}* heterozygotes are labelled by antisera against *t^{w32}*, *t^{w5}*, *t⁰* and *t^{w18}* haplotypes. The important point is that each of these *t* haplotype associated antigens is expressed at the morula stage. Thus it seems that although there seem to be *t*-stage specific lesions in development, there is no temporal correlation in the expression of the *t^{*}* antigens. There is, presumably no sequential switching on of genes coding for surface products which carry these antigens and which have been hypothesised to control important developmental events.

The evidence for the developmental

stage specificity of the *t* lethals has been largely derived from morphological studies of developing homozygous embryos *in utero*. The general interpretation has been that the *t* alleles affect specific cell types within the embryo. Recent work by Wudl and Sherman (*Cell* 9, 523; 1976) has suggested that some *t* mutations are general cell lethals and do not affect a single cell type. When individual *t^{ws}/t^{ws}* embryos are cultivated *in vitro*, all the different cellular derivatives die; control explants of normal embryos survive favourably in these conditions. Wudl and Sherman also claim that the *r^s* mutation may be a simple cell lethal, although Erickson and Pedersen (*J. exp. Zool.* 193, 377; 1975) have obtained different results. Later acting *t¹⁸* mutations exhibit more specific cell lethality since homozygous embryos will develop in ectopic sites, are histologically anaplastic, but devoid of mesodermal derivatives (Artzt & Bennett *J. natn. Cancer Inst.* 48, 141; 1972). Thus it is possible that there are two classes of *t* mutations, those which are lethal for all embryonic cell types and those which have cells that can escape the homozygous condition.

Whether or not the molecules carrying the *t* antigens are involved in cellular interactions in development remains an open question. The complexity of the cellular specificity of *t* mutations, the *T/t* locus genetics and the antisera make the task of answering it difficult. The isolation in culture of cell lines carrying particular *t* haplotypes will avoid the problems associated with sperm cytotoxicity and eventually allow biochemical characterisation of these antigens as with the F9 (Jacob *Immun. Rev.* 33, 3; 1977). The identification of another antigen, PCC4, expressed by multipotent embryonal carcinoma and spermatozoa, but only on the inner cell mass of the newly implanted blastocyst (Gachelin *et al. Devl Biol.* 57, 199; 1977) lends support to the view that surface changes are co-incident with important determinative events in the embryos. □

Food lost to pests

from Robert M. May

FOR crops of grain in mediaeval times, a rough rule of thumb said that of every three grains, one was lost to pests or in storage, one was for next year's seed, leaving one to eat. The yield from modern crops is much greater, and the fraction to be set aside for seed is negligible, but the fraction lost has changed surprisingly little.

Drawing together information compiled by the US Department of Agriculture, Pimentel (*Bull. Ent. Soc. Am.*

Migration of a spreading axis

from Peter J. Smith

THAT part of the East Pacific rise which separates the Pacific plate to the west from the Nazca plate to the east between 0°S and 34°S is the most rapidly spreading section of the world oceanic ridge system. Some of it is also among the most complicated insofar as there may be two very small crustal plates lying between 22°S and 26°S and between 32°S and the triple junction at 34°S, respectively (Herron *Nature phys. Sci.* 240, 35; 1972). Elsewhere, however, the spreading régime appears to be fairly simple, not least in the area around 31°S which has recently been investigated by Rea (*Earth planet. Sci. Lett.* 34, 78; 1977). Here the topographic axis of the rise is now continuous and almost linear. But as Rea has discovered, it has not always been so.

Evidence for past deviations comes chiefly from the magnetic anomalies in the region. The axial anomaly is straight as is that associated with the Matuyama-Gauss reversal at 2.41 Myr. But the anomalies associated with the Olduvai event at 1.73 Myr and the Brunhes-Matuyama reversal at 0.7 Myr are curved. Moreover, in the southern part of the survey area the Jaramillo event anomaly at 0.92 Myr is offset about 10 km right laterally. Thus although the spreading régime may be simple now and may have been simple more than 2 Myr or so ago, things were evidently more complicated between times.

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Rea interprets these data in terms of local migrations of the spreading axis, following Blakely (*Geology* 3, 35; 1975) who invoked a similar phenomenon to explain spreading rate variations in the northeast Pacific. The axis of the East Pacific rise at 31°S was straight about 2.41 Myr ago but then gradually became more and more concave to the east until it broke in such a way that the southern section was offset to the west compared with the northern section. The break soon healed, however, leaving a continuous, but curved, axis which gradually became straight again. Throughout much of this period the spreading was also asymmetric (greater towards the east) and the total spreading rate varied.

The details of these axial migrations are, of course, specific to the area under investigation; but the underlying processes are more general. It appears to be significant, for example, that when the axial offset occurred, the result was not to move the axis further in the direction of curvature but to return it to a more central position, presumably closer to the underlying magma source. In fast spreading areas the relatively thin lithosphere cannot confine the spreading centre as easily as can the much thicker lithosphere in slow spreading regions, and so the axis tends to waver. When the axial curvature becomes too severe, however, it is relieved by offsetting which reduces the geographical deviation between the ridge axis above and the magma source below.

22, 20; 1976) has shown that in the US in 1974 the losses in agriculture to insects were 13%, to diseases 12%, and to weeds 8%. This sums to 33% of the annual crop lost to pests, broadly defined. Corresponding average annual losses to pests throughout the decade 1951–1960 were 13% to insects, 12% to diseases, and 9% to weeds, and throughout the decade 1942–1951, 7% to insects, 11% to diseases, and 14% to weeds: over this span of time, losses to insects and plant diseases increased, while losses from weeds decreased.

Although the overall fraction of US crops lost to insects has increased despite the application of insecticides, important reductions of insect losses have been made on some crops. Thus

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the fraction of the potato yield lost to insects has declined from around 22% in 1910–1935 to 16% in 1942–1951 and 14% in 1951–1960. In contrast, losses in the apple crop (mainly due to codlin moth and apple maggot) in the same time intervals have been 10%, 12% and 13%; this reflects both decline in cultural controls formerly practised in orchards and increased emphasis on cosmetic aspects of marketing. For corn, a major grain crop, insect losses increased from around 4% in 1942–1951 to 12% in 1951–1960: contributing factors appear to be the continuous cultivation of corn on the same land year after year (increasing susceptibility to rootworm), and the planting of corn types that are less resistant to insect depredations. In all, about 1.2 billion pounds of pesticides (insecticides, herbicides, fungicides), or about 6 pounds per person, are at present

used annually in the US. These pesticides are not distributed uniformly among all crops; the non-food crops of cotton and tobacco for example, account for 50% of all insecticides used in agriculture.

Of particular interest are the patterns of control with pesticides compared with the broad range of bioenvironmental controls, which include cultural manipulations (such as crop rotation), natural enemies, and genetic resistance. Pimentel's survey of the USDA data shows that for insect pests, bioenvironmental controls are used on about twice as many acres as insecticidal methods (9% compared with 5%). For plant pathogens, some form of bioenvironmental control is used on 90% of acreages compared with only 0.5% on which fungicides are used. Crop rotations have been, and continue to be, a major control method for many crop diseases; the absence of a crop for a year or so can cause the population of its pathogens in the soil to die out. For weeds, the estimate is that bioenvironmentally controlled acreage beats that treated with herbicides by 80% to 15%. The relation between the life-history strategy of a pest and the best way to control it is a fascinating one, and the subject of much contemporary research (see *Nature* 264, 211; 1976).

In the US, crop losses from birds and mammals are small (estimated around 1%), although they can be significant for particular crops, such as cherries. Post-harvest losses are, however, substantial. Such losses of stored food to insects, rodents, and microorganisms are estimated around 9%. Adding pre- and post-harvest losses, we see that in the US a total of about 39% (33% plus 6%) of the total food supply is lost to pests.

For the world as a whole, crop losses to pests have been estimated to be about 35% (Cramer, in a journal deserving full citation, *Pflanzenschutz-nachrichten* 20, 1; 1967). This includes destruction by insects, pathogens, weeds, mammals, and birds. The latter two categories of loss are more severe in the tropics and sub-tropics than in temperate regions, but the first three categories remain predominant. Pimentel observes that available evidence tends to suggest that the "green revolution" technology has intensified losses due to pests; recent strains that have been bred with more attention to natural resistance to insects and pathogens may help reverse this trend. Annual world use of pesticides runs around 4.1 billion pounds, or 1 lb per person, with 34% of the total accounted for by North America, 45% by Western and Eastern Europe, and 21% by the rest of the world.

A rough estimate of world post-harvest losses is 20% (Pimentel *et al. Science* 190, 754; 1975), ranging from 9% in the US to 40% and 50% in some of the developing nations in the tropics. Adding these to the pre-harvest losses, an estimate of 48% (35% plus 13%) is obtained for the total food loss. While it may be argued that some of these estimates are a bit on the high side, the fact remains that pest populations (broadly defined) are consuming or destroying something like one-third to one-half of the world's food supply. This is a loss we can afford less and less as time goes on. □

The Earth's core

from C. Hazard

A RECENT paper in *The Moon* (16, 41; 1976) by R. A. Lyttleton must be of considerable interest to geophysicists. The hypothesis that the Earth's core consists mainly of molten iron that drained to the centre quite early on in the Earth's history is so familiar that few seem ever to question its validity. That the core is liquid is demonstrated by the absence of shear waves in it, and that it is metallic is suggested by the requirement of a conducting medium for the electric currents proposed to account for the magnetic field. That the material is iron was conjectured largely on the belief that iron is the only heavy element sufficiently abundant to explain the high density in the core, and implicit in this is the assumption that the core-mantle boundary represents an abrupt change in chemical composition.

However, there is the less well popularised alternative hypothesis that the Earth is fairly undifferentiated throughout and that in fact the core-mantle discontinuity is a change in phase from a non-metallic solid state to a denser metallic liquid state maintained by the great pressure and high temperature within the core. That is, it is a second kind of phase change involving a much greater density increase than the recognised so-called 20°-discontinuity, which occurs at a depth of 413 km where the change is to a different crystalline form.

When the widely held belief was that the Earth in its early stages was molten throughout, the iron core hypothesis seemed to follow as a natural consequence of the heaviest components settling to the centre, but now that cosmogony points to an origin as

a cold body accreting from planetesimals in the original gas-dust solar nebula the phase-change hypothesis has obvious attractions. However, since the late 1940s when proposed by Ramsey (see *Mon. Not. R. Astr. Soc.* 110, 325; 1950), it has found little favour with geophysicists and geologists, although to the best of my recollection it used to be discussed favourably by P. M. S. Blackett in his undergraduate lectures at Manchester in the early 1950s. In recent years the hypothesis has been revived, and its consequences worked out in great detail by R. A. Lyttleton.

According to Lyttleton's development (*Proc. R. Soc.* A275, 1; 1963; *Proc. R. Soc.* A287, 471; 1965) of Ramsey's hypothesis, the Earth formed at sufficiently low temperature to be solid throughout. Then radioactive heating gradually raised the temperature of the central regions to a critical value (of a few thousand degrees K), at which time the catastrophic collapse in a matter of minutes demonstrated mathematically by Ramsey's theory resulted in the sudden formation of a large metallic liquid core. From known seismic data, this core has been shown by Lyttleton to have had a radius just over 2,000 km. Under the influence of continuing radioactivity it has grown steadily to its present radius of 3,473 km, with an accompanying decrease in the overall radius of the Earth of slightly more than 0.1 mm per year, enough to diminish the overall radius by nearly 400 km in 4.5 eons!

It is known that hydrogen just about doubles its density and goes over to a metallic form when subjected to a pressure only about half that at the core-mantle boundary but there is no direct evidence that terrestrial material could also undergo such a transformation. However, in his recent paper in *The Moon*, Lyttleton has produced a persuasive argument in support of the phase-change theory by showing that the contraction required on this hypothesis can quite naturally remove the well-known inconsistency in the tidal theory of the evolution of the lunar orbit, first encountered by Jeffreys in the 1920s and which has remained unresolved ever since (Jeffreys *Mon. Not. R. Astr. Soc.* 80, 309; 1920; *The Earth* edns 2-6, Cambridge University Press, 1920). Jeffreys endeavoured to account for the apparent secular accelerations of the Moon and Sun solely in terms of the lunar and solar couples resulting from tidal friction, but found that the actual observed ratio, 2.9, of these accelerations was far lower than the tidal-theory value of about 7.0. It is this discrepancy that Lyttleton now shows can be removed by introducing terms making allowance for a secularly decreasing moment of inertia of the

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Earth, and that the required rate of increase is in remarkable agreement with the previously calculated rate of decrease of the Earth's radius (*Proc. R. Soc. op cit.*; 1965).

Small as the rate of decrease may seem, it yet gives a change in the Earth's angular velocity comparable to that produced by the tidal couples, but of opposite sign and therefore accelerative as required. Lyttleton shows that while a similar but much smaller acceleration could also be produced by separation of iron into the core, such draining down is generally regarded as having occurred largely, if not entirely, early in the Earth's history, and even under the most favourable assumptions as to the present rate, it could not nearly account for the observed discrepancy. It would seem that unless the adherents of the iron core hypothesis can produce a comparably satisfactory alternative explanation for this discrepancy emerging from the standard Jeffreys tidal theory, Lyttleton has struck a telling blow for the phase-change hypothesis for the nature of the core. □

Beaded bubbles

from Robert W. Cahn

AN infinite monocrystalline element containing a second element in solid solution, not liable to any phase transformation, tends to a spatially uniform distribution of the dissolved element, and diffusion will persist until that stable terminal state is attained. That cautious opening sentence incorporates several provisos: if the solid consists of distinct crystal grains or, being less than infinite, is bounded by free surfaces, complications ensue. In general, solutes tend to segregate either to or away from free surfaces or grain boundaries; improved methods of microanalysis have allowed a considerable body of experimental information to be assembled concerning this process of 'Gibbsian' segregation (J. H. Westbrook, in *Interfaces Conference, Melbourne 1969* (ed. Gifkins, R. C., 283 (Butterworth, 1969); E. D. Hondros, *ibid.*, page 77). Hondros's extensive experimental work has led him to the conclusion that solutes with a small equilibrium bulk solubility (usually associated with very different solvent and solute atomic sizes) are particularly apt to segregate to interfaces and surfaces, thereby relieving lattice strain in the solvent. As Westbrook points out,

surface segregation is not restricted to external surfaces; it can apply equally to internal pores, such as are found in imperfectly sintered powder compacts. Westbrook emphasises the role of the binding energy that holds vacancies and solute atoms together; if this is high, any excess vacancies diffusing to a pore must drag solute atoms along with them.

Materials scientists responsible for the design of nuclear fuel elements are currently much concerned with pores—'voids' is the preferred term—in neutron-irradiated metals and ceramics. During irradiation (especially of the metal used to encapsulate the fuel) interstitial atoms and vacancies are formed in equal numbers and diffuse away to 'sinks', predominantly dislocations and grain boundaries. These sinks attract interstitial atoms more effectively than vacancies and the surplus vacancies then assemble to form small voids, visible in the electron microscope. The voids cause swelling of the material, a disease which must be kept within strict bounds for safety reasons. The principal strategy for minimising swelling is to choose suitable base metals and to maximise the concentration of effective sinks for interstitials and vacancies alike. Now, another strategy has been identified.

Some years ago, P. R. Okamoto and H. Wiedersich of the Argonne National Laboratory (*J. nucl. Mater.* **53**, 336; 1974) observed indications that solute elements segregate to radiation-induced voids in a stainless steel. The evidence was indirect—micrographic features indicating pronounced lattice distortion alleged to accompany the segregation—and it was not possible to identify the segregating species. The investigators suggested that it was interstitial solute atoms generated by the irradiation which segregate and lead to the high lattice distortion observed in the electron microscope. Thus Hondros's view that segregation acts to reduce lattice strains is not always in accord with observation.

The whole matter has now been clarified by an elegant set of experiments published by K. Farrell, J. Bentley and D. N. Braski of Oak Ridge National Laboratory (*Scripta Met.* **11**, 243; 1977). They irradiated aluminium foils to very high neutron doses, enough to generate about 0.4 atomic % silicon by $^{27}\text{Al} \rightarrow (\text{n}, \gamma) \rightarrow ^{28}\text{Al} \rightarrow ^{28}\text{Si} + \text{e}$. When the foils were thinned and examined by scanning transmission electron microscopy (STEM), they were seen to contain not only ordinary voids and silicon precipitates, as expected, but also some anomalous voids sitting on the surface of the foil or extending beyond the foil edge. In fact, these voids, bounded by a dark margin, were inhabiting empty space. Very

detailed electron microprobe experiments using the ultrafine STEM beam proved that these free-standing voids were coated with shells (4–11 nm thick) of (possibly amorphous) silicon. The aluminium surrounding them had been dissolved away during the thinning process, leaving hollow silicon spheres standing clear of the foils. Silicon has a small solubility in aluminium, and meets Hondros's criterion for ready Gibbsian segregation.

Farrell, Bentley and Braski point out that this substantial Gibbsian segregation offers the hope of influencing the growth rate of voids, especially in their early stages. The segregated solute must alter the specific surface energy at the void walls and thus affect the efficacy of the void in attracting vacancies—and hence must alter the swelling rate too. If Okamoto and Wiedersich are right in presuming that radiogenic interstitial solutes are peculiarly prone to Gibbsian segregation, then it may be possible to design alloys with initial solutes which, after radiation-induced transmutation, segregate to the incipient voids and inhibit their further growth. That would be an elegant way of setting one consequence of irradiation to neutralise another. □

The plasmopause revisited

from Michael J. Rycroft

WITHIN the charged particle environment of the Earth, which encompasses the ionosphere and magnetosphere, the plasmopause is a physically significant boundary. The plasmopause marks the boundary between the cool, relatively dense plasma of terrestrial origin and the hot, tenuous plasma of both terrestrial and solar wind origin which resides in the outer magnetosphere. This demarcation feature is aligned along lines of force of the Earth's magnetic field; the feet of these field lines touch the Earth's surface at near 60° magnetic latitude. They extend out into space, crossing the equatorial plane at a geocentric radial distance of ~4 Earth radii. Such field lines are termed $L \sim 4$ field lines, using the parameter L introduced by McIlwain as a co-ordinate to order observations of the van Allen radiation belt electrons.

The existence of the plasmopause was discovered independently by two different scientists almost 20 years ago.

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Using observations made at Seattle and Stanford, D. L. Carpenter, of Stanford University, California, was studying whistlers. Audio-frequency electromagnetic radiation from lightning discharges, which penetrates the lower ionosphere and is guided from one hemisphere to the other by ducts of enhanced plasma density, which are themselves field-aligned, suffers dispersion. The characteristic signature of the whistler in the frequency-time domain is thus explained. As in much of geophysics, the received signal is the result of integration of a weighted varying physical parameter over the propagation path, and has to be interpreted either by comparison with model computations or by inversion. Using the former method with multipath whistlers, Carpenter showed that the plasma density profile in the equatorial plane decreases smoothly out to $L \sim 4$. There the profile often exhibits a 'knee'; the plasma density decreases sharply, within a fraction of an Earth radius, by an order of magnitude or more, to only $\sim 10^7 \text{ m}^{-3}$. (Carpenter's knee is now termed the plasmopause). He also showed that the plasmopause persisted for several hours at least, and that its L -value was a maximum at dusk, at the bulge in the plasmopause.

K. I. Gringauz, of the Space Research Institute of the USSR Academy of Sciences, Moscow, was studying measurements made by "charged particle traps" carried on the Luna (Lunik) 1 and 2 rockets launched to the Moon. These can be interpreted to yield positive ion density values at intervals along the spacecraft trajectory. With coworkers, he found that the plasma density could suddenly decrease markedly on the outgoing part of the trajectory.

Thus these *in situ* observations confirm the 'remote sensing' whistler observations. The results obtained by one technique complement those obtained by the other. The power of one method (low cost and good temporal coverage for example) is offset by the serendipity of whistler occurrence and their time-consuming analysis. The power of the other method (unambiguous measurement at a point in space) is offset by the high cost of satellite experiments and the fact that many hours—or even days—pass between successive observations at neighbouring points on a particular L -value. In the intervening time, the plasma density may have changed—by an order of magnitude—due to dynamical processes.

A symposium on the Physics of the Plasmopause was held in Trieste in September 1974, under the aegis of the European Geophysical Society. In a special issue of the journal *Annales de*

invited review and contributed papers were published. The subjects covered ranged from peculiar features of the mid-latitude ionosphere associated with the plasmopause to energetic charged particle phenomena, and included treatments of geomagnetic pulsations observed at $L \sim 4$ and of some relevant theoretical problems.

The variations of the L -value of the plasmopause with changing geomagnetic activity (which follows changes of parameters of the solar wind) and at different local times have been investigated by several authors. Most recently N. C. Maynard and J. M. Grebowsky, of the NASA Goddard Space Flight Center, Maryland, have performed such a study based on observations taken aboard the Explorer 45 (or S³-A) satellite. It is curious and, in retrospect, unfortunate that this satellite did not carry an instrument to measure the plasma density directly. However, an instrument to measure the d.c. electric field responds, at low plasma densities, to potentials associated with the Debye sheath around the satellite. As the satellite moves in its 7 h 49 min orbit outwards in the equatorial plane, towards its apogee at a geocentric distance of 5.24 Earth radii, it crosses the plasmopause; there the instrument becomes saturated due to these sheath potentials. Using coincident ground-based whistler and Explorer 45 observations, M. G. Morgan and Maynard (*J. geophys. Res.* **81**, 3992; 1976) showed that saturation occurred at a plasma density of $6 \times 10^7 \text{ m}^{-3}$, to within a factor of two.

In a recent paper, 'The plasmopause revisited' (*J. geophys. Res.* **82**, 1591; 1977), Maynard and Grebowsky have studied statistically the plasmopause position. They have used observations taken over 15 months and covering 1972 completely. The local time coverage was from 2300 LT, through noon to 0800 LT. They found that the plasmopause bulge occurs at 2000 or 2100 LT at geomagnetically quiet times, but migrates towards 1800 LT as magnetic activity increases. During quiet periods, a secondary bulge near local noon is found, confirming results obtained recently by K. I. Gringauz and V. V. Bezrukikh.

Observations of the variation of the plasmopause position with both local time and geomagnetic activity have been interpreted in recent years in terms of the electric field within the magnetosphere. This results from the superposition of the electric field representing the corotation of the inner magnetospheric plasma with the Earth, 'tied' by the geomagnetic field lines, and the electric field resulting from the flow of the solar wind past the magnetosphere which drives the convection

from the night-side to the day-side in the equatorial plane. This latter field may be at least partially 'shielded' from the day-side of the Earth, for example by the shorting-out action of the highly conducting, sunlit ionosphere or by the build-up of space charge layers. The fact that the bulge moves towards dusk, with increasing magnetic activity, indicates that this shielding becomes less effective for the larger convection electric fields occurring then. The secondary bulge in the plasmopause near noon can be explained by the projection of the Sq dynamo electric field, in the ionosphere, along geomagnetic field lines into the magnetosphere.

Maynard and Grebowsky note that the plasmopause L -value is rather better ordered when plotted against Dst , the index of ring current intensity, than when plotted against K_p , the three-hourly, global (planetary) index of geomagnetic activity. This may be explained either by the plasmopause controlling the loss of ring current ions, by way of an ion-cyclotron resonance (loss-cone) instability, or by both the plasmopause and the energetic ring current ions responding together to changes of the convection electric field.

For the future, work remains to be done in the realm of plasma physics, to improve our understanding of the behaviour of the plasmopause as a boundary layer between cool and hot plasmas. The importance of the plasmopause in controlling wave-particle interactions, between whistlers and energetic electrons for example, or between ion-cyclotron waves and multifarious ions, is becoming recognised more and more. Now is the era when we can test the hypotheses that we have developed for the Earth's magnetosphere by applying them to exciting new observations of planetary magnetospheres. Whether or not we have to make reappraisals will remain to be seen. □

A hundred years ago

ON FRIDAY night a series of interesting experiments with the Jablochhoff electric light took place at the West India Docks, under the direction of M. Denayrouze. The apparatus used for the occasion consisted merely of an electro-magnetic machine worked by a small steam-engine, some insulated wires, and the electric candles, which are the invention of M. Jablochhoff, and composed, as we have already described, of two carbons placed side by side with a slip of insulating substance between them, which burns away with the carbon exactly in the same way as the wax of a wax candle is consumed with the wick.

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review article

The 'universal' dielectric response

A. K. Jonscher*

A review of dielectric data for a wide range of solids proves the existence of a remarkable 'universality' of frequency and time responses which is essentially incompatible with the multiplicity of currently accepted detailed interpretations. Certain unique features of the universal behaviour strongly suggest the dominant role of many-body interactions.

THE dielectric response of solids has been the subject of extensive investigations for the best part of a century. Existing data cover an enormous range of frequencies from 10^{-6} to 10^{16} Hz, neither limit being absolute. When it comes to theoretical interpretations, the properties of gases and liquids at microwave to optical frequencies are the subject of powerful modern approaches, while the technologically most important response of solids at sub-GHz frequencies remains the subject of piecemeal approaches tailored to individual materials and often based on highly arbitrary models and analytical techniques. In this paper I propose to review some of the available experimental evidence on the frequency, time and temperature dependence of the dielectric parameters for a wide range of solids and to establish the existence of a very general or 'universal' pattern of behaviour which should provide a framework for the classification of a wide range of dielectric phenomena. I will then try to demonstrate the inadequacy of the existing theoretical treatments and I shall propose a novel approach based on many-body interactions. I shall be concerned exclusively with the frequency range below 1 GHz where phonon and quantum phenomena are negligible.

Definitions

A vacuum capacitor with an electric field E between its metallic plates has an interfacial charge $Q_0 = \epsilon_0 E$, where $\epsilon_0 = 10^7/4\pi c^2 = 8.845 \times 10^{-12}$ F/m is the dielectric permittivity of free space and c is the speed of light. If the field varies with time, the charge Q_0 follows exactly, there is no 'inertia' in the vacuum response. If the capacitor is filled with a material medium—gaseous, liquid or solid—the induced charge is increased by the polarisation P of the medium

$$Q = Q_0 + P = \epsilon_0(1 + \chi)E = \epsilon E \quad (1)$$

where ϵ is the dielectric permittivity and χ the susceptibility of the medium.

Since no material medium can follow instantaneously the variations of the field, there is a delay between the polarisation and the field. It is convenient to define the time response to a step-function field, $E(t) = 0$ for $t < 0$, $E(t) = E_0$ for $t > 0$, of the current

$$i(t) = dP/dt = \epsilon_0 E_0 f(t) \quad (2)$$

where $f(t)$ is the dielectric response function. The 'vacuum response' is represented as a δ -function at $t = 0$, but it cannot be resolved experimentally. The response of polarisation to an arbitrarily time-varying signal $E(t)$ is given by the convolution integral

$$P(t) = \epsilon_0 \int_0^\infty f(\tau) E(t-\tau) d\tau \quad (3)$$

which indicates that the system retains 'memory' of past excitations. In a harmonically varying field with radian frequency $\omega = 2\pi f$, f being the circular frequency in Hz, Fourier transformation of equation (3) gives the frequency dependence of polarisation

$$P(\omega) = \epsilon_0 \chi(\omega) E(\omega) \quad (4)$$

where $P(\omega)$ and $E(\omega)$ are Fourier components of $P(t)$ and $E(t)$, respectively, and $\chi(\omega)$ is the complex Fourier transform of $f(t)$

$$\chi(\omega) = \chi'(\omega) - i\chi''(\omega) = \int_0^\infty f(t) \exp(i\omega t) dt \quad (5)$$

where $i = (-1)^{1/2}$. The real component χ' gives the component of polarisation in phase with the field, the imaginary component, known as the dielectric loss gives the quadrature component. In a field of peak amplitude E_0 the energy lost per radian is $\epsilon_0 \chi''(\omega) E_0^2/2$, and the power lost is $\sigma(\omega) E_0^2/2$, and this defines the alternating current (a.c.) conductivity

$$\sigma(\omega) = \sigma_0 + \epsilon_0 \omega \chi''(\omega) \quad (6)$$

showing the relationship between a.c. conductivity and dielectric loss, with the direct current (d.c.) conductivity, σ_0 , being included. Equation (5) shows that $\chi'(\omega)$ and $\chi''(\omega)$ are inter-related and they are, in fact, Hilbert transforms¹ of one another, known in this context as the Kramers-Kronig relations which are valid in the most general conditions, subject only to the linearity of response. It follows that any one of the three functions $\chi'(\omega)$, $\chi''(\omega)$ and $f(t)$ fully determines the response of the system and the other two may be derived from it.

Mechanisms of polarisation

Of the several possible polarisation mechanisms, induced atomic, or electronic, and also ionic lattice polarisations, respond so rapidly, that they are effectively instantaneous below GHz frequencies, contributing a purely real value, ϵ_∞ , to the permittivity. Permanent molecular dipoles, ionic defects of dipolar type (vacancy-interstitial pairs) and also slowly mobile hopping charge carriers of electronic, polaronic or ionic nature produce much slower responses so that the permittivity of a medium containing some or all of these may be written in the form

$$\epsilon(\omega) = \epsilon_\infty + \epsilon_0 \sum_\alpha \chi_\alpha(\omega) \quad (7)$$

where the index α labels the various mechanisms and χ_α are complex in the sense of equation (5).

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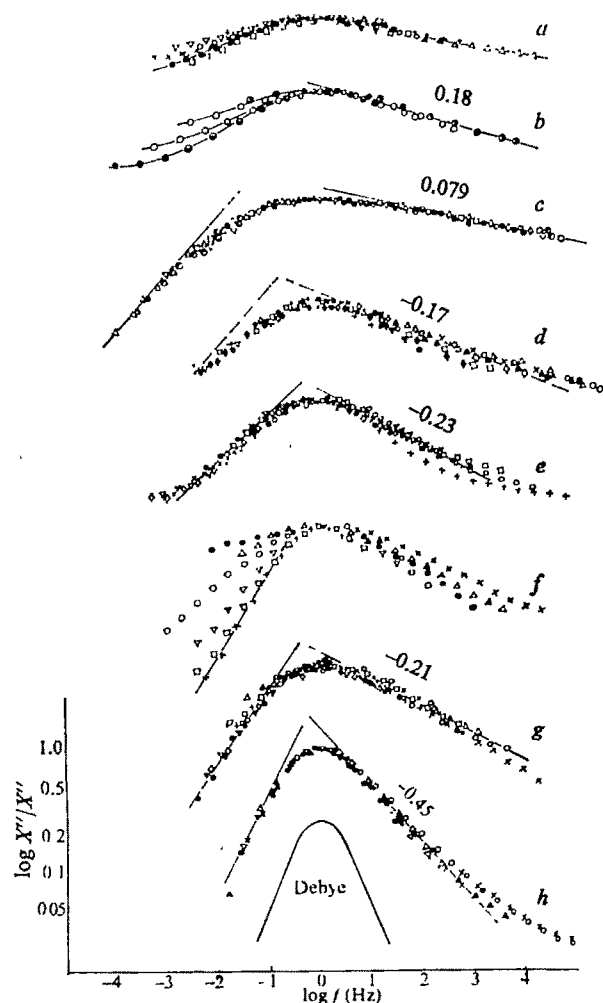


Fig. 1 A compilation of dielectric loss data for a range of polymeric materials. The points marked with different symbols correspond to data taken at different temperatures and transposed laterally to make the loss peaks coincide. The vertical scale is $\log \chi''$ and the consecutive graphs are displaced vertically for clarity. The numbers indicate slopes of the straight line portions of the graphs. *a*, Low temperature data for polychloroprene; *b*, PCTFE 12% crystalline; *c*, polyethylene terephthalate; *d*, polymethyl methacrylate; *e*, high temperature data for polychloroprene; *f*, polyethyl methacrylate; *g*, polydian carbonate; *h*, polyvinyl acetate. The shape of a pure Debye peak is also included for comparison. (From ref. 5).

Dipolar responses

The classical case of dielectric response is that of a set of identical non-interacting dipoles free to rotate against some viscous resistance in a fluid-like medium—Debye's original model³—or 'jumping' by thermal excitation between two preferred orientations separated by a potential barrier—a situation more likely to be found in solids. The Debye susceptibility is given by the expression

$$\chi(\omega) \propto \frac{1}{1+i\omega\tau} = \frac{1}{1+\omega^2\tau^2} - i \frac{\omega\tau}{1+\omega^2\tau^2} \quad (8)$$

where the relaxation time τ is generally thermally activated and the loss peak frequency varies with temperature as

$$\omega_p = 1/\tau = \nu_0 \exp(-W/kT) \quad (9)$$

where ν_0 is some 'attempt-to-jump' frequency and W is an activation energy. The loss peak given by the second term in equation (8) is symmetric on a $\log \omega$ scale and has a width at half height of 1.144 decades.

Typical examples of dipolar behaviour may be found in many polar polymers³ in which the dipolar species may be well characterised both in type and in density. However, the dielectric loss in real polymers departs strongly from the Debye response and some examples are shown in Fig. 1 giving data corresponding to various temperatures 'normalised' by the customary displacement along the frequency axis to align the peaks^{4,5}. The Debye response is shown for comparison. The sharper α peaks are observed above the glass transition temperature, T_g and have approximately twice the width of Debye, the lower-temperature β peaks are hardly recognisable.

The main point I wish to emphasise here is that the post-peak response of all these materials may be characterised by the relation

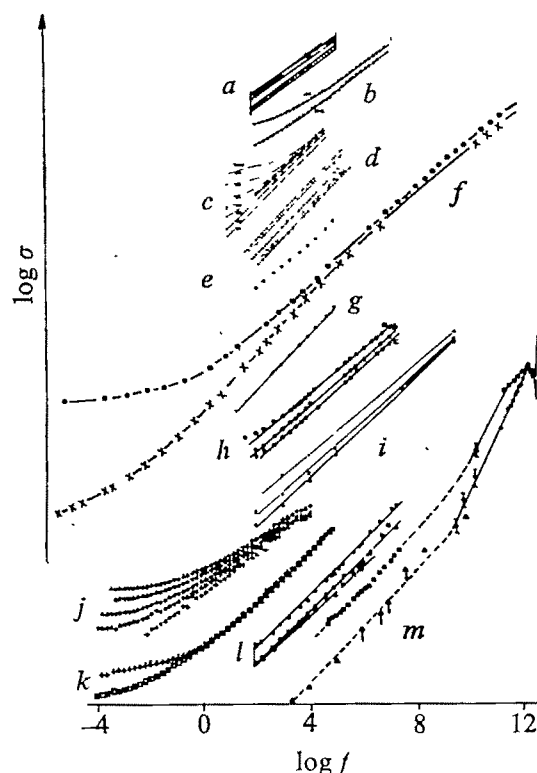


Fig. 2 The compilation of a.c. conductivity data for a range of materials arranged on a common $\log f$ basis (in Hertz) but displaced vertically along the $\log \sigma$ axis for clarity. Data sets denoted by one letter are on a common $\log \sigma$ scale. *a*, Single crystal silicon in the impurity hopping range by electrons at (bottom to top) 3.0, 4.2, 8.0 and 12.0 K. (ref. 13); *b*, single crystal β alumina at 77 and 87 K—a classic fast-ion conductor by Na^+ ion movement¹⁴; *c*, glow-discharge deposited amorphous silicon in the temperature range 84–295 K (ref. 15); *d*, a range of chalcogenide glasses at 293 K, top to bottom: Sb_2S_3 , $\text{Sb}_{30}\text{As}_{10}\text{S}_{60}$, $\text{Sb}_{30}\text{As}_{30}\text{S}_{40}$, As_2S_3 , $\text{Sb}_{10}\text{As}_{30}\text{S}_{60}$ (ref. 16); *e*, single crystal anthracene at 294 K with 1 M saline solution as contact—two sets of measurements are shown (not resolvable on the scale of the present diagram)¹⁷; *f*, single crystal anthracene (—x—x—) and evaporated β carotene (---) at 294 K (ref. 18). The two sets of data are on corresponding $\log \sigma$ scale, showing the close similarity of the results in the a.c. range, with significant departures in the d.c. levels. These results show the superposition of a very shallow peak at 10^6 Hz with an underlying trend $\sigma \propto \omega$; *g*, trinitrofluorone-polyvinyl carbazole (TNF PVK) (ref. 19); *h*, three glasses (a) 50 P_2O_5 –50 FeO , (b) 50 P_2O_5 –40 FeO –10 CaO , (c) 50 P_2O_5 –25 FeO –25 CaO : room temperature²⁰; *i*, 80 V_2O_5 –20 P_2O_5 glass at three temperatures²¹; *j*, evaporated amorphous SiO_x (silicon monoxide) in the range of temperature (bottom to top) 211–297 K (ref. 22); *k*, stearic acid 9-layer film between Al and Au electrodes in the dark ($\square\square$) and in the presence of ultraviolet light ($++$) (ref. 23); *l*, three amorphous samples (top to bottom) As_2Se_3 , Se and As_2S_3 . The measurements, taken at 300 K are believed to be those of the bulk material and not to be influenced by the electrodes²⁴; *m*, two samples of As_2Se_3 at room temperature²⁵ extending to far-infrared frequencies and showing a steeply rising ω^2 region above 10 GHz, characteristic of lattice vibrational processes.

$$\chi''(\omega) \propto \omega^{n-1} \quad \text{with } n < 1 \quad (10)$$

or by a superposition of two such functions with the higher-frequency component having values of n closer to unity. The exponents n are weakly temperature dependent, decreasing with increasing temperature. Many polymeric materials, polar as well as non-polar, show very flat losses over many decades of frequency, with superimposed very weak peaks, a behaviour consistent with $n \approx 1$ and not at all compatible with Debye-like responses.

A particularly interesting class of dipolar materials are clathrates⁶ in which 'guest' molecules are individually enclosed in cages of H_2O molecules having almost spherical symmetry. These materials show the same type of 'non-Debye' behaviour given by equation (10). There are also examples of solutions of polar molecules in non-polar solvents giving the same response not consistent with any obvious interpretation⁷.

Carrier polarisation

This category covers a very wide range of mechanisms and materials, the one common feature being that the charge carriers involved move by discontinuous hopping movements between localised sites—they may be electrons, polarons or various ions. Electrons or polarons normally hop between sites randomly distributed in space and in energy⁸ but it is almost impossible to distinguish between them experimentally. The d.c. conductivity is determined by the most difficult hops in percolation paths between the two electrodes, a.c. conductivity is thought to arise from more limited displacements, with two-centre hopping said to constitute the high-frequency limit. By contrast, ions move typically over much smaller, nearest-neighbour distances and it is particularly interesting to note, therefore, that neither the magnitude of the a.c. conductivity, its activation energy nor, in particular, its frequency dependence can be taken as reliable guides to the nature of the dominant carrier responsible for polarisation.

Electronic hopping is associated primarily with the class of amorphous and glassy semiconductors⁹⁻¹² which have received a good deal of experimental and theoretical attention in the last fifteen years or so. Figure 2 shows a selection of conductivity data for several of these materials¹³⁻²⁶, plotted on a common scale of $\log f$ and displaced vertically along the $\log \sigma$ (ω) scale for clarity. The same figure also shows some other materials not necessarily associated with glassy electronic semiconductors, notably β alumina, the classic ionic conductor, as well as anthracene and β carotene.

The frequency dependence of the electrical conductivity of all these very different materials may be represented by the expression

$$\sigma(\omega) \propto \omega^n \quad n \leq 1 \quad (11)$$

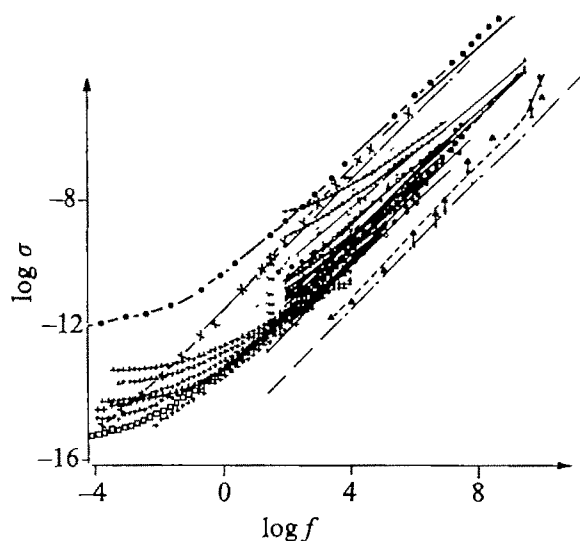


Fig. 3 The compilation of the data for the a.c. conductivity of Fig. 2 on a common scale of $\log \sigma$ ($\Omega \text{ cm}$)⁻¹ showing the relatively narrow range of a.c. conductivity for a very wide range of materials. The upper and lower sloping chain-dotted lines correspond, respectively, to dielectric loss ($\chi'' = 10$ and 10^{-3} , independent of frequency).

which is fully equivalent to (10). Most of the samples show no sign of d.c. conductivity or even of any deviation from a single value of the exponent n , while some show a smaller value of n at lower frequencies—*b*, *c* and *k*—yet others give a d.c. or near-d.c. dependence—*j* and *k*. The temperature dependence of the a.c. conductivity is generally much weaker than that of the corresponding σ_0 , the exponent n showing the same trend as in Fig. 1. Both β carotene and anthracene reveal a shallow loss peak superimposed on the general trend and centred on 1 MHz. The characteristic feature of all the other data is the absence of any evidence of loss peaks which would correspond to slopes significantly in excess of unity on the low-frequency side.

The numerical values of the exponents n for the different materials are listed in Table 1. The one conclusion that may be drawn is that values in the range $0.5 < n < 0.9$ are closely associated with proven carrier transport: hopping electrons in crystalline and amorphous silicon, SiO_x and stearic acid, the response of which is shown in more detail in Fig. 4. In this case it has been established that the values of n close to unity are to be associated with the 'lattice' response²⁴. This distinction between 'lattice' and 'carrier' responses corresponding, respectively, to 'intrinsic', that is irreducible, and

Table 1 Numerical values of the exponent n for different materials in Fig. 2

Diagram	Material	Temperature range (K)	Exponent n	Remarks
<i>a</i>	Single X-tal Si	3.0–12.0	0.79–0.74	
<i>b</i>	Single X-tal β alumina	77–87	0.75–0.70	high frequency
<i>c</i>	a Si	84–255	~ 0.5	low frequency
<i>d</i>	$\text{Sb}_2\text{As}_{2-x}\text{S}_3$	293	1.0	
<i>l</i>	As_2Se_3	300	1.0	
	Se	300	0.95	
	As_2S_3	300	0.92	
<i>m</i>	As_2Se_3	300	0.92	
<i>e</i>	Single X-tal anthracene	294	0.85	
<i>f</i>	Single X-tal anthracene	294	~ 0.95	shallow peak
	β carotene	294	~ 0.90	shallow peak
<i>g</i>	TNF-PVK		~ 1.0	
<i>h</i>	$\text{P}_2\text{O}_5\text{--FeO--CaO}$ glass	300	0.85–0.89	
<i>i</i>	$\text{V}_2\text{O}_5\text{--P}_2\text{O}_5$			
<i>j</i>	SiO_x	211–297	0.6–0.7	
<i>k</i>	Stearic Acid	300	0.95	high frequency
			0.6–0.7	low frequency

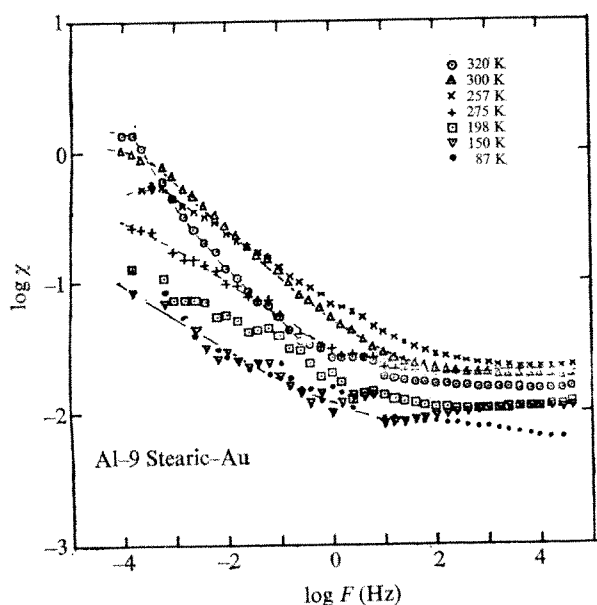


Fig. 4 The frequency dependence of the dielectric loss of a 9-layer structure of stearic acid between an aluminium base electrode and a gold top electrode, for a range of temperatures. From ref. 26.

'extrinsic' processes due to some impurities or injected carriers, may prove to be a useful diagnostic tool in the assessment of dielectric materials²⁷.

The expanded data of Fig. 2 are replotted again in Fig. 3 but this time on a common scale of $\log \sigma$, in order to bring out the very narrow range of values of $\sigma(\omega)$ at any particular frequency. The two sloping chain-dotted lines correspond to $\chi'' = 10$ and 10^{-3} independent of frequency, that is they cover four powers of ten and virtually all the data fall in this range, while a large majority actually fit into two decades, between 1 and 10^{-2} . This narrow range of the observed values of the loss was pointed out by me some time ago²², when I speculated on the possible role of contacts, as I found it difficult to believe that all these diverse materials should show such similar bulk properties. However, I do not think that there can be much doubt about the fact that we are dealing with genuine bulk properties and this is further confirmed by the very similar range of absolute values of dielectric loss in many polymeric solids.

Other dielectric materials

Many more examples may be quoted of dielectric behaviour following the general trend given by equations (10) and (11), with either a loss peak or a trend towards d.c. at low frequencies and a few of these will be mentioned here: sputtered films of lead-zinc-niobate and other materials^{29,30}; electrolytic capacitors of Al or Ta on plain foil, deeply etched foil or sintered porous bodies, thus proving that the geometrical configuration of the films has no significant influence on the response (F. Meca, personal communication); aqueous solutions of alkali halides, for example LiCl (ref. 31); dry sand follows equation (11) over seven decades with $n = 0.84$ (ref. 32); a wide range of composite dielectric materials such as rubbers, ceramics, sintered alumina, insulating glasses^{33,34}, and so on.

The same 'flat' frequency dependence of loss is found in some ferro-electric materials such as lead-zirconate-titanate and similar ceramics and likewise triglycene sulphate (TGS). In TGS the results are particularly striking since the Curie temperature, T_c , is at 50 °C and both χ'' and χ' show a singularity at T_c as functions of T , both being fairly flat in frequency at all temperatures, while the ratio χ''/χ' remains almost constant across the ferro-electric-para-electric transition (D.C. Dube, personal communication).

Very thin films also sometimes display the response characterised by equation (10). It is found down to monomolecular layers of, for example, stearic acid²⁶. Silicon P-N junctions form capacitors in one of the most perfect dielectrics known and it is therefore particularly interesting that their loss shows very shallow peaks resembling the flatter examples of dipolar losses in Fig. 1 (ref. 35). Interesting dielectric properties are shown by the solid electrolytes, defect solids in which ions show very high mobilities owing to the fact that there is no need to create the vacancies normally required for ionic conduction³⁶⁻³⁸. Many of these materials show negligible electronic conduction. Their electrical properties are commonly measured as complex impedance over a range of frequencies and the complex impedance diagrams are circular arcs with centres depressed below the real axis. I have pointed out that this is a direct consequence of a frequency-dependent conductivity of the type given by equation (11) and detailed studies confirm that this behaviour is found in a wide range of materials, single crystalline such as the β alumina in Fig. 2, polycrystalline and glassy (J. M. Reau, personal communication).

Time-domain response

Subject to the linearity of response, for example absence of charge injection¹⁰, the response function $f(t)$, equations (2) and (5), corresponding to the 'non-Debye' response given by equation (10) is

$$f(t) \propto t^{-n} \quad (12)$$

and most dielectric materials show this form of current response to step-function excitation which is known as the Curie-von Schweidler law⁴¹⁻⁴⁴. Where a loss peak is present, as in Fig. 1, the response becomes⁴⁵

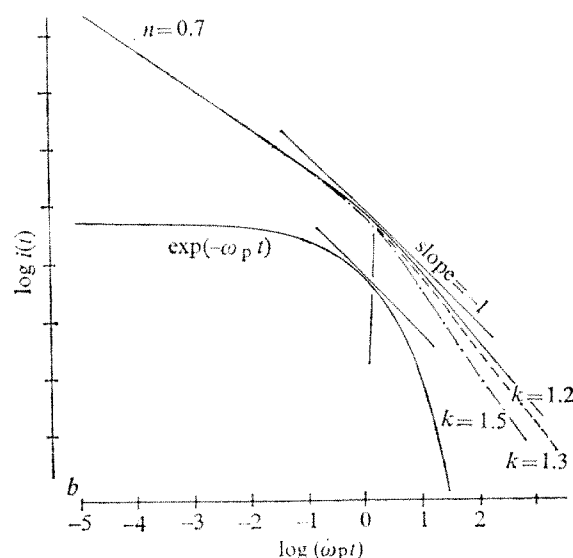
$$f(t) \propto [(\omega_p t)^{1+m} + (\omega_p t)^n]^{-1} \quad (13)$$

where $m < 1$, and this is represented in Fig. 5. Physically this corresponds to two consecutive processes, the Curie-von Schweidler law with the negative exponent $n < 1$ for times $t < 1/\omega_p$, and a similar law but with the negative exponent $1+m$ in excess of unity at times $t > 1/\omega_p$.

The 'universal' law of dielectric response

The dielectric response of almost all solid dielectrics—and of some liquid ones as well—may be represented by either of

Fig. 5 Current response under step-function excitation according to equation (13), with $n = 0.7$ and $k = 1+m = 1.2, 1.3$ and 1.5 . The slope becomes equal to -1 at $\omega_p t = 1$. The Debye response $\exp(-\omega_p t)$ is also shown for comparison. From ref. 45.



the two diagrams of Fig. 6; *a* represents the classical dipolar response modified by the experimentally observed broadening, while Fig. 6*b* represents the 'carrier' dominated low-frequency response. The common feature is the extended range of frequencies over which the loss obeys the law given by equation (10), which has the unique property that its Kramers-Kronig transform has the same functional form but for a constant factor, so that the real part of susceptibility is the same function of frequency and the ratio

$$\chi''(\omega)/\chi'(\omega) = \cot(n\pi/2) = \text{const.} \quad (14)$$

independent of frequency. This is in complete contrast with the Debye behaviour for which this ratio is equal to $\omega\tau$.

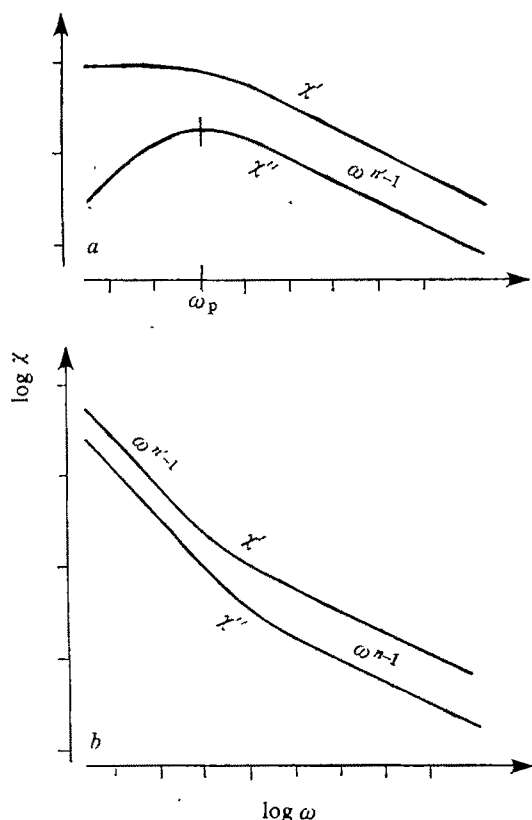
I define this as the 'universal' dielectric response and its range of validity extends from ω_p , in those cases where it is observable, to the onset of phonon and quantum phenomena at around 1–10 GHz. In many materials we have an unbroken range of six to ten decades of frequency, while in others it is easy to recognise the superposition of two mechanisms of this type but with different values of n .

As well as the range of frequencies, what is most remarkable is the range of material for which this universal law is applicable:

All structural forms: single crystal, polycrystalline, amorphous and glassy, aggregates; **all types of chemical bonds:** covalent, ionic, molecular; **all possible types of polarising species:** dipoles, hopping electrons, ions; **a wide range of geometrical configurations:** 'bulk' samples, thin films down to molecular dimensions, intricate geometries.

These facts have been known, albeit perhaps not in their full extent, for many years, some going back seventy years and

Fig. 6 Schematic representation of the two types of dielectric response in the frequency domain, plotted as the logarithm of the real and imaginary components of the susceptibility against the logarithm of frequency. Both show the 'universal' behaviour with ω^{n-1} dependence at the higher frequencies. Diagram (*a*) corresponds to a dipole-dominated response, with a loss peak at the ω_p , diagram (*b*) gives case carrier-dominated low-frequency response with a different value of the exponent $n' < n$. The scale markers signify units on the logarithmic scales.



many attempts have been made to interpret this behaviour in terms of some model or other, according to the particular class of materials under consideration. A more detailed critique of this will be found elsewhere (A.K.J., to be published) and I have to limit the discussion to bare minimum here. The dipolar responses are frequently interpreted in terms of distributions of Debye-like relaxation processes, although it is difficult to see how the required range of τ , covering eight to ten decades of time can be justified in any particular system. Hopping electrons and polarons have been the subject of very extensive treatments^{46–55}, in terms of percolation theories and jump probabilities between states randomly distributed in space and in energy. While these treatments are very plausible and mathematically much more refined than many other dielectric theories, it is difficult to believe that the range of materials shown in Fig. 3 should exhibit such closely similar absolute values, given the wide range of physical hopping parameters likely to be involved. Moreover, the applicability of the universal law down to the thinnest samples presents some difficulties in this approach, where consecutive hops are needed to give the observed characteristics.

Ionic conductors are being interpreted in terms of some distributions of 'relaxation times' which are not specified and therefore are difficult to criticise. Alternatively, it is postulated that the bulk behaves in a frequency-independent manner, that is as an ideal dielectric in parallel with an ideal conductance, while the entire frequency dependence derives from limitation of ion supply across interfacial barriers^{56,57} whose transmission coefficients have to be postulated according to need. However, these coefficients are not separately measurable so they remain conjectural and, in any case, it is difficult to explain by this means values of n in the range 0.5–0.8 typically found in many ionic conductors.

Attempts have been made to move away from Debye philosophy by introducing diffusion-limited relaxations^{58–60}, but apart from a certain lack of physical clarity regarding the mechanism, it is only possible to explain by this means values of n close to 0.5. Diffusion is also at the base of the Warburg impedance mechanism⁶¹ commonly used in liquid electrolyte problems and often invoked in solid electrolytes, although its applicability here is suspect since it requires the presence of two mobile ionic species with opposite signs of charge.

Many analytical attempts have been introduced to explain the time-domain Curie-von Schweidler law in terms of correlation functions^{62–64} but again one is left with an essential arbitrariness at some stage in the choice of the functions required to give agreement with experiment.

Many-body interactions

I suggest that, faced with the sheer universality of the observed behaviour, in terms not only of a common frequency dependence but, not least, of a narrow range of absolute values of loss in very diverse materials, one is left with the overriding impression of an essential insufficiency of all these different interpretations and the need for an entirely fresh approach, based on a principle of sufficient inherent generality to be capable of embracing all the materials under consideration.

What is even more remarkable is that the universality does not stop with dielectric responses—it is well established in the case of mechanical moduli of disordered systems, both polymeric⁶⁵ and metallic. Here I refer not to the well known fact that dielectric and mechanical loss peaks follow similar trends⁶⁶, but to the much more significant, in the present context, relaxation law beyond the peaks which is of the form (10), even though the exponent n is not the same in both cases. There is also a well established equivalent of the Curie-von Schweidler law in mechanical relaxation.

There is even some evidence, although much less well documented, that magnetic losses in many ferrites follow an almost frequency-independent trend at low frequencies⁶⁷, thereby tentatively extending the observed universality of the dynamic relaxation to the third fundamental stress-strain relationship.

I have suggested that the universally observed constancy of the ratio (14) implies the physically elementary fact that⁵

$$\frac{\text{energy lost per radian}}{\text{energy stored}} = \cot(\pi/2) = \text{const.} \quad (15)$$

independently of frequency and that this should be taken as the universal criterion of dielectric, mechanical and, possibly even magnetic relaxations in solids.

In the case of dielectrics, I have noted that the one general property of all systems under consideration is that the polarisation is due to discontinuously hopping electrons or ions, or to likewise discontinuously jumping dipoles and I have introduced the simple concept of screening of charges and dipoles. This screening is the result of many-body interactions and has the simple and universal property that it adjusts 'slowly' to the 'fast', that is effectively discontinuous, jumps of individual particles or dipoles. I have suggested that this 'dual' time scale leads to the property (15) and, therefore, inescapably through the Kramers-Kronig relations, to the universal frequency dependence (10).

The introduction of many-body formalism into the interpretation of the universal dielectric response of solids does find a striking parallel in the theory of X-ray singularities in metals⁶⁸ and also in the problem of infrared divergence⁶⁹, both leading to theoretical expressions for time-dependent responses resulting from many-body interactions which are identical with the Curie-von Schweidler law. While the time scales in question are separated by many orders of magnitude, the similarity of the resulting power-law dependence on time invites a detailed examination of the validity of this approach. (This study is currently being carried out in collaboration with K. L. Ngai of the Naval Research Laboratory, Washington DC.)

An attempt to explain the conductivity law (11) in terms of many-body interactions was made by Pollak and Pike⁷⁰, who associated it with the low-temperature specific heat anomaly in glasses. An even earlier suggestion to this effect was made by Garton⁷¹ but seems to have been largely forgotten. These are clear pointers in the direction of many-body interactions being an acceptable explanation of the universal dielectric response, but a great deal of work requires to be done before the situation could be regarded as clarified. I am suggesting that the dielectric response provides us with an 'analogue solution' of a many-body interaction and this may prove valuable in the theoretical studies. In addition, I believe that the physical significance of the energy criterion (15) in the frequency domain should not be underestimated—it may give us a vital insight into the processes in question, mechanical no less than dielectric. My screened-hopping model is based on this criterion, but there may exist other possible ones.

The loss peak and other low-frequency processes

To the extent to which we may accept the many-body approach, through the energy criterion, as explaining the universal response in the frequency domain, equation (10), in the range in which it is valid, we need an interpretation of the loss peaks which are found in dipolar systems. I suggest that their interpretation in the accepted 'distribution' argument is not very satisfactory, not least in view of the fact that the pre-exponential factor ν_0 in equation (9) is found to cover the prodigious but physically meaningless range 10^3 to 10^{30} Hz, for both mechanical and dielectric cases (C. Lacabanne, personal communication), where 10^{13} would have been expected as a 'lattice' frequency.

I have suggested that a very simple explanation of loss peaks in the context of our many-body approach may be found in the time-domain picture of Fig. 5 (ref. 45). The loss peak arises from the transition in the time-domain response after a step function excitation, from a 'primary' relaxation process with $n < 1$, to a 'secondary' process with a larger exponent which completes the adjustment to the steady state. The primary

process is the Curie-von Schweidler law, corresponding to the energy criterion in the frequency domain. The secondary process is not at present understood, although its physical necessity in the case of dipolar polarisation follows from the requirement to have a finite total charge in the system. There is, however, nothing inherently implausible in a concept of a succession of two different processes in a many-body relaxation. The important point is that the loss peak is the resultant of this transition from one process to another and that the loss peak frequency is determined solely by the time of transition. That this time should be activated with a well defined energy, as is normally observed, seems perfectly understandable—it must be related to some thermal processes, but there is no need to associate the pre-exponential factor ν_0 with any particular 'lattice' frequency, as is the case in the Debye philosophy.

The apparent absence of loss peaks in carrier-dominated situations, as well as the presence in some cases of strong low-frequency dispersions obeying an approximately $1/\omega$ law for both $\chi'(\omega)$ and $\chi''(\omega)$ presents an interesting problem but lies beyond the scope of the present treatment.

Conclusions

Experimental evidence leads to the conclusion that there exists for a wide range of solids a universality of dielectric behaviour demanding a much more unified interpretation than the hitherto accepted specialised approaches which are somewhat arbitrary and which are tailored to the various separate classes of materials. Moreover, the universality extends beyond the dielectric properties—it is well established in the case of dynamic mechanical response of glassy solids and there is tentative evidence for its presence in magnetic response.

I suggest that the unified approach should be based on the indisputable common property of all condensed matter—the many-body interactions between its constituent parts.

The otherwise somewhat arbitrary functional form of the universal response has the unique property in the frequency domain that the ratio of energy lost per cycle to energy stored is independent of frequency and I am proposing to make this the physical criterion of all dielectric responses. The corresponding time dependences are identical with those found theoretically for a number of many-body interactions, such as the X-ray singularity and the infrared anomaly.

In the dielectric case this criterion is satisfied by mutual screening of hopping charges or dipoles and is based on the relatively slow adjustment of screening in comparison with the practically instantaneous hopping transitions of individual charges or dipoles. A new interpretation is proposed in the same framework of the peaks of loss observed in dipolar systems.

This work was carried out under financial assistance from the Science Research Council and from Chelsea College. I am grateful to many colleagues for helpful criticisms and to some for permission to quote unpublished information. I am particularly indebted to Dr. K. L. Ngai for his comments on the many-body interactions.

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articles

Turning points in Phanerozoic history

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During the last 450 Myr there has been concomitant variation in the Earth's rotation rate, the polarity bias of the geomagnetic field, the amount of activity at ocean ridges, sea level and climate. At turning points when trends in the variables reverse themselves, climatic instabilities have disrupted biological ecosystems and led to mass extinctions.

ONE of the most interesting cooperative efforts within the earth sciences is that between palaeontologists and geophysicists in which skeletal growth rhythms recorded in fossils are used to elucidate the Earth's rotational history. It has been established that the rotation rate has fluctuated during the Phanerozoic. This synthesis suggests that subtle long term variations in other parameters can be correlated with changes in rotation rate.

Rotational history

The modification of skeletal growth by external environmental cycles can produce within a shell a detailed record of daily, fortnightly, monthly and annual events. The potential of such structures was first fully realised by Wells¹ who made counts on Middle Devonian corals which indicated that at that time, 390 Myr, there were 400 solar days in the year. As Wells^{1,2} appreciated the slowing down of the Earth's rate of rotation, which this result indicated, corroborated the geophysical prediction that the lunar tidal torque has a braking effect by which angular momentum is transferred from the Earth to the Moon. Soon afterwards Scrutton³ who also used Middle Devonian corals, made counts indicating that there had been 30.6 d in the synodic month and Runcorn⁴ showed how this pair of observations could be used to test for possible changes

in the Earth's moment of inertia. Runcorn's analysis, though probably unreliable due to the statistical uncertainty inherent in the original data^{5,6}, suggested that any changes in the Earth's moment of inertia must have been small and subsequent workers have generally accepted tidal friction as the operative mechanism in the rotation deceleration. However, as more palaeontological data became available it became evident^{7,8} that there had not been a uniform rate of decrease in spin velocity and a significant recent advance was made by Creer⁹ who recognised that there have been periods of earth history when the Earth's rate of rotation has accelerated (Fig. 1). During the last 500 Myr these periods of acceleration have alternated with periods of deceleration of spin velocity and there are turning points of two types, firstly spin maxima at transitions from acceleration to deceleration and secondly spin minima at transitions from deceleration to acceleration. Revised estimates indicate that spin maxima occurred 65 Myr (end Cretaceous) and 375 Myr (middle Upper Devonian) ago and that spin minima occurred 235 Myr (end Permian) and 445 Myr (late Ordovician) ago (Fig. 1). The periods of acceleration recognised by Creer⁹ cannot be easily explained by tidal friction alone and suggest that some additional mechanism or mechanisms must be operating. This additional component seems to be a fluctuating system superimposed on a general trend for deceleration of spin velocity which presumably represents the effects of tidal friction (Fig. 1).

Creer⁹ also recognised that the turning points were closely associated in time with periods of instability in the palaeomagnetic field (Fig. 1). While periods of rotational acceleration are characterised by a predominance of normal polarity and periods of rotational deceleration are characterised by a predominance of reversed polarity, turning points are associated with phases of mixed polarity which show no obvious polarity bias and which may also have

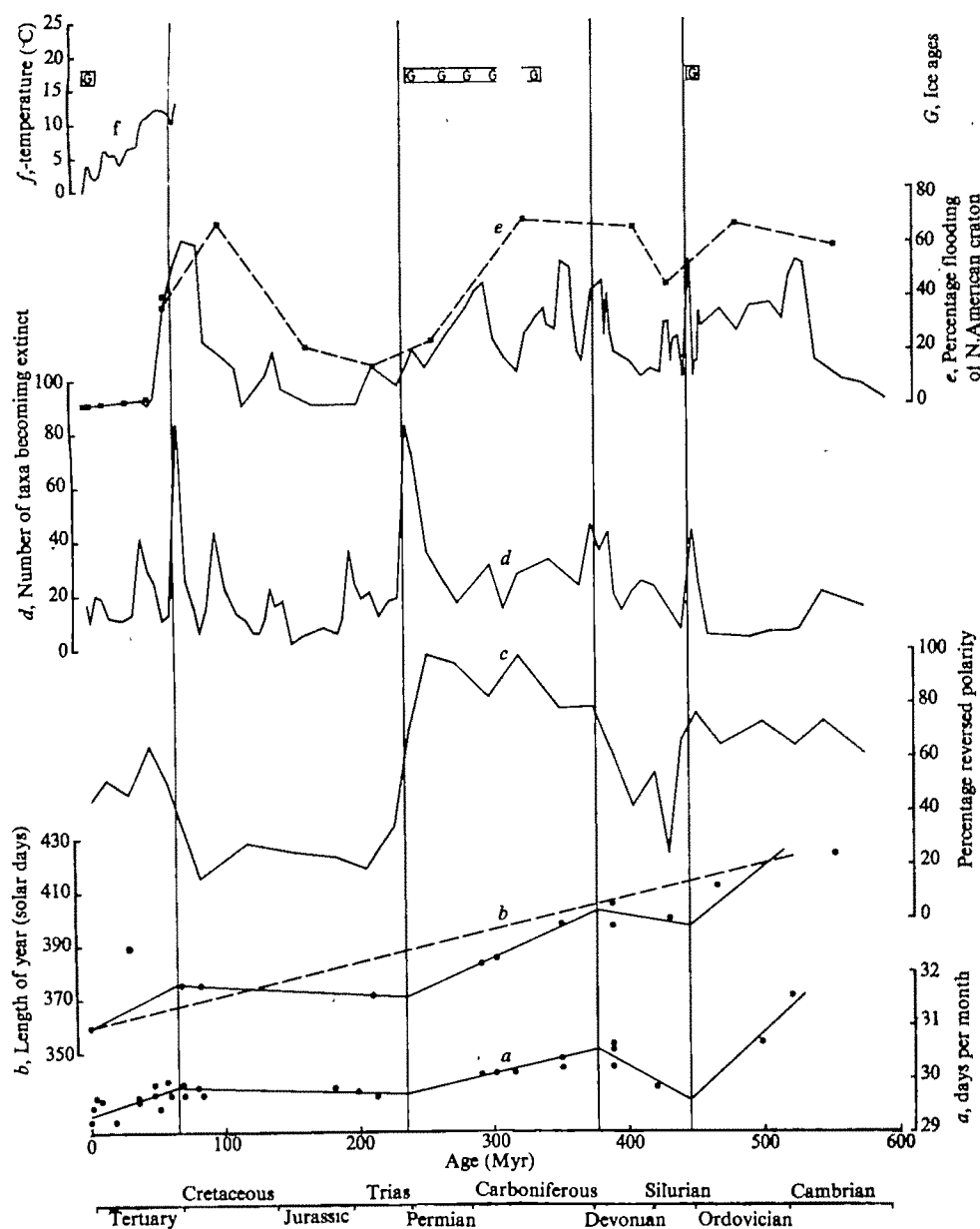


Fig. 1 Aspects of Phanerozoic history. *a*, Number of solar days per month (modified from Creer⁴ using palaeontological data⁷⁻¹³). *b*, Number of solar days per year (modified from Creer⁴; using palaeontological data 1, 2, 8, 14; the dashed line is extrapolated from the present rate of deceleration¹⁵). *c*, Percentage of reversed polarity (based on Creer⁴). *d*, Number of higher taxa, of all biological groups, becoming extinct (based on Cutbill and Funnell¹⁶). *e*, Percentage flooding of the N. American craton (based on Wise¹⁷; the dashed curve is a period-by-period smoothed curve). *f*, Temperature of high latitude marine waters (based on Savin *et al.*¹⁸). *g*, Occurrence of ice ages (refs 43, 45-47).

had high reversal frequencies. A close link between the Earth's rotation and its magnetic field has long been recognised: there seems to have been a near alignment of rotational and dipole axes throughout time and it has been suggested that the influence of rotational forces on the fluid outer core may be vital to the generation of the magnetic field¹⁶. On the other hand the irregular decade fluctuations in the Earth's rate of rotation have been correlated with changes in the geomagnetic field and attributed to the transfer of angular momentum between the core and the mantle¹⁷. The core-mantle coupling is generally interpreted as being an electromagnetic effect though viscous forces and topographic features of the boundary may also be important¹⁸. Though extraterrestrial sources cannot be altogether discounted, the geologically detectable rotational and magnetic fluctuations are most probably due either to changes in the Earth's moment of inertia¹⁹ or to an internal redistribution of angular momentum¹⁸ and it is a great pity that the palaeontological data are not yet sufficiently precise to allow elimination of one or other of these possibilities using an extended form of Runcorn's analysis.

In the middle Carboniferous and again during the Late

Jurassic and early Cretaceous there were periods in which the palaeomagnetic field became moderately disturbed¹⁹. However, unlike the conditions at turning points, the field retained an overall polarity bias and there was no associated switch in the direction of the predominant polarity. One might predict that these events were accompanied by slight inflections in the rate of change of the Earth's rotation but the palaeontological data are at present too scanty to allow this possibility to be tested. These episodes could perhaps be interpreted as aborted turning points and it is worth noting that they are followed by the most important Phanerozoic quiet intervals namely the Permian-Carboniferous (Kiaman) Reversed Interval and the Cretaceous (Mercanton) Normal Interval. The Cenozoic magnetic field has apparently always been relatively unstable but there seems to have been within the last 10 Myr a change from a predominance of reversed polarity¹⁹ (Fig. 1) and during the same period there has also been an increase in the frequency of reversals²¹. Astronomical estimates of the present rate of secular deceleration give a value lower than that which seems to have operated during the last 65 Myr (Fig. 1) and this, together with the magnetic evidence, might indicate that we are at or close

to another spin minimum. Including this putative turning point and the two aborted ones it seems that turning points are separated by a characteristic time interval with an average length of 74 Myr.

Mass extinctions

Several investigations²² of Pleistocene deposits have noted a significant correlation between magnetic reversals and extinctions of fossil species and these results are consistent with Uffen's hypothesis²³ that during reversals biological systems would be adversely affected by increased radiation doses. Simpson²⁴ extended this hypothesis to suggest a correlation between mass extinctions and periods of high reversal frequency and this is strongly supported by Creer's data⁵ since the major mass extinctions in the late Ordovician, middle Upper Devonian, end Permian and end Cretaceous coincide with turning points (Fig. 1). Considerable doubt²⁵⁻²⁷ has been cast on whether a magnetic reversal alone would cause a sufficient increase in radiation fluxes but it has recently been suggested that if a solar flare occurs during a reversal it might cause the ozone layer, which shields the Earth's surface from ultraviolet radiation, to become depleted²⁸. Alternative theories²² have suggested a direct relationship between the magnetic field and the well-being of organisms or an indirect relationship through climatic effects.

Correlations between climatic changes and changes in the intensity of the geomagnetic field have been demonstrated and possible linking mechanisms have been discussed^{29,30}. As previously noted there is a correlation between geomagnetic field intensity and rotation rate¹⁷ and Lambeck and Cazenave⁶⁷ have detected a correlation between climate and rotation rate. They have however calculated that the magnitude of the rotational changes is not sufficient to fully explain the associated climatic effects. It is suggested here that magnetic fluctuations cause climatic effects partly as a direct effect and partly through the associated changes in spin velocity, and that the magnetic instabilities at turning points cause climatic instabilities.

The general pattern of mass extinctions is for the communities of stable environments with their great diversity of specialised member species to be most severely affected³¹. Thus offshore benthic communities were more affected than onshore benthic communities^{32,33} and that most complex of all marine associations, the reef community, underwent at each mass extinction a dramatic collapse from which it often took millions of years to recover^{34,35}. The climatic instabilities at turning points were thus probably only triggers, which by causing a few extinctions and/or a reduction in primary production, created a house of cards effect leading to the collapse of ecosystems³¹.

Neither the mid-Carboniferous nor the end Jurassic events were associated with abnormally high extinction rates but Late Pleistocene and Recent extinctions, though dominantly affecting large (that is, specialised) terrestrial vertebrates, are well documented^{36,37}. While man must in all probability take a large share of the blame for these extinctions it may well be that he is only exacerbating an unstable situation.

Climatic history

Apart from the possible climatic instability at turning points the periods of acceleration seem to be characterised by climatic amelioration while periods of deceleration are characterised by climatic deterioration. Thus spin minima were preceded by ice ages with marked seasonal and latitudinal climatic contrast (Fig. 1) while spin maxima were preceded by climatic optima with an equable warm climate. Isotopic evidence^{38,40} (Fig. 1) shows clearly the temperature decline that has taken place, especially at high

latitudes, during the Tertiary. Mesozoic isotope data^{41,42} are less well controlled but indicate warm temperatures even at high latitudes and, though there were fluctuations a trend for temperature increase up to the Late Cretaceous optimum about 80 Myr ago. The Permo-Carboniferous and the Late Ordovician Ice Ages are well documented⁴³ and a recent synthesis³⁹ has suggested that there was a reduction in climatic gradients during the Silurian and Devonian. The Cretaceous optimum gave rise to the acme of reef development by scleractinian corals and the early Upper Devonian optimum was similarly the acme of the tabulate coral and stromatoporoid reef assemblage³⁴. The scleractinia as a group have declined during the Tertiary³⁴ and the poor representation of reef corals in the Carboniferous and Permian may also be a climatic effect. As in the Permian, bryozoans and sponges are an important element of Ordovician carbonate build-up³⁴ formed during a phase of climatic deterioration. A pre-mass extinction diversification has been detected in the Cretaceous and in the Devonian but not in the Permian⁴⁴ which may be a consequence of its different climatic regime.

It is interesting to note that Upper Palaeozoic ice sheets seem to have developed before the mid-Carboniferous aborted event⁴⁵⁻⁴⁷ and this may explain the long time span of the Permo-Carboniferous Ice Age.

Climate and eustasy

Though a change in the length of the day would, for instance, cause a small change in the diurnal temperature inequality⁴⁸, the overall trend evident in the rotational data suggests that the link between climate and spin velocity is not a direct one. Rotational changes may have been accompanied by other changes in the Earth's orbit, which could also have caused slight climatic effects, but the most important control on climate would seem to be sea level⁴⁹. Hays and Pitman postulated⁴⁹ that increased climatic contrast will result from eustatic lowering of sea level and that eustatic raising of sea level will cause 'moderation and stabilisation of climate'. The global Mesozoic transgression and the subsequent Cainozoic regression are well documented⁴⁹⁻⁵³ (Fig. 1). Sea level changes in the Palaeozoic are less clear and estimates based on N. American data⁵⁴ are clearly affected by tectonic events in the Appalachians. Cruder curves⁵³ (Fig. 1) suggest that there may have been overall transgression from the Late Ordovician to the Late Devonian followed by a regression into the end of the Permian. The Ashgill regression⁵⁴ and the widespread Ordovician-Silurian unconformity⁵⁵ represent the effects of the Late Ordovician eustatic minimum and in the Devonian a series of increasingly penetrative transgressions, which reach a maximum in the Late Devonian, have been recognised⁵⁶. Ice ages are thus a consequence of the increased climatic contrast resulting from regression but the exact timing of the onset of glaciation and the subsequent glacial history will be affected by other factors such as distribution of land masses, atmospheric turbidity and orbital motions. The association of glaciation with aridity³⁷ fits well with this theory and the increasing severity of Pleistocene glacial periods³⁷ can be linked to the continued decrease in sea level.

Though orogenic events can cause eustatic changes in sea level⁵² the dominant eustatic control seems to be the volume of oceanic ridges which is itself controlled by plate spreading rates (refs 49, 50, 58 and 59). Thus increased plate generation not only leads to an increasing volume of ocean ridges, a raising of sea level and an amelioration of climate but also correlates with an acceleration of mantle spin velocity and a predominance of normal polarity. Fast Cretaceous and slow Cenozoic spreading rates have been deduced for a number of ridge systems^{49,59}. More detailed work, despite uncertainties due to imprecision of the polarity time scale, suggests that Tertiary spreading rates

decreased¹ gradually up to about 10 to 15 Myr ago, since when there has been a slight increase in spreading rate^{60,61}. This may again suggest an impending turning point. The possible role of tidal and rotational forces in the plate tectonic mechanism has been discussed by several workers⁷²⁻⁷⁵, and, whether the relationships are causal or not, clearly ridge activity and changes in mantle spin velocity and predominant polarity are all related to some unifying process.

A coherent picture—but gaps still remain

Though much work needs to be done to improve our knowledge of the factors considered here, the data present a coherent picture. The Cenozoic, the Late Devonian to Permian and the Ordovician were times of deceleration of mantle spin velocity, of predominantly reversed polarity, of decreasing oceanic ridge activity, of global regression and of climatic deterioration leading to ice ages. By contrast in the Silurian to early Upper Devonian and in the Mesozoic there was acceleration of mantle spin velocity, predominance of normal polarity, increasing ocean ridge activity, global transgression and climatic amelioration. Turning points between phases of different type are marked by magnetic instability, possible climatic instability and by mass extinctions. Aborted turning points occurred in the middle Carboniferous and late Jurassic. Despite the many imperfections and biased nature of the geological record a subtle but distinctive background pattern can thus be detected in it. Whatever the cause may be this pattern is not only a record of surface processes but also of deep seated events some of which may even have taken place within the Earth's core.

This synthesis has a number of important implications. First, it places important new constraints on Earth models. These must be considered especially in discussions of the origin of the geomagnetic field, the mechanisms affecting the Earth's rotation and the driving force for plate tectonics. Second, the pattern can be applied to the Precambrian as an aid to understanding events and processes in the Proterozoic (Whyte, in preparation). Finally it allows us to make some simple predictions about the future geological history of the Earth. Thus not only do we seem to be at present at or close to a turning point (spin minimum) but we can predict that it will be followed by a period lasting about 60 to 90 Myr in which there will be rotational acceleration, a dominantly normal magnetic field, increasing ridge activity, global transgression and climatic amelioration.

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Biogenic lipids in particulates from the lower atmosphere over the eastern Atlantic

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Higher plant wax is demonstrated in aeolian dusts, showing the potential of aeolian transport in contributing significant amounts of terrestrial organic lipids to the marine environment over geological time periods.

The importance of aeolian particulate transport from the continents to the marine environment has been examined mainly through investigations of mineral and trace element contents of sediments and particulate matter in the water column^{1–4}. This transport mechanism, however, has been

Table 1 General aeolian dust collection data and sample descriptions

Sample*	Date collected	Ship's tracks†	Dust colour	Dust loading ($\mu\text{g m}^{-2}$)	Wind system	Potential dust source
1	Apr. 72	34°44'N, 13°38'W 28°30'N, 15°54'W	Brown	0.18	Westerlies/ N.E. Trade Boundary	N.W. Europe N. Africa
2	Mar. 71	21°41'N, 17°41'W 14°04'N, 18°07'W	Beige	36	N.E. Trades	Sahara
3	July 72	17°47'N, 17°42'W 19°24'N, 17°40'W	Beige	~20	N.E. Trades	Sahara
4	Dec. 74	03°44'N, 12°00'W 08°50'N, 15°44'W	Grey-brown	9.6	N.E. Trades	Sahara
5	Dec. 74	01°14'S, 08°01'W 03°44'N, 12°00'W	Dark grey	0.4	N.E. Trades	Sahara
6	July 72	07°12'S, 04°02'W 02°37'S, 07°42'W	Brown-black	0.18	S.E. Trades	Southern Africa
7	July 72	11°52'S, 00°29'W 07°12'S, 04°02'W	Dark brown	0.08	S.E. Trades	Southern Africa
8	Sept. 74	09°40'S, 01°31'W 14°42'S, 02°06'E	Dark brown	1.4	S.E. Trades	Southern Africa
9	July 72	22°26'S, 07°58'E 17°01'S, 03°30'E	Brown	0.29	S.E. Trades	Southern Africa
10	Sept. 74	14°42'S, 02°06'E 19°26'S, 05°47'E	Beige-brown	2.0	S.E. Trades	Southern Africa
11	Nov. 71	29°03'S, 13°22'E 25°06'S, 09°59'E	Black	5.6	S.E. Trades	Ships
12	July 72	30°03'S, 31°03'E 24°24'S, 24°07'E	Grey-brown	4.0	Variables	Southern Africa

*Samples 1 (TAF 5), 3 (TAF 71), 6 (TAF 65), 7 (TAF 64), 9 (TAF 62) and 12 (TAF 57) see reference 10; 2 (E2) see ref. 11; 4 (DC 2), 5 (DC 1), 8 (DC 4) and 10 (DC 5) Cross, unpublished results; and 11 (AH 3) see ref. 12.

†Indicates the position of the ship at the start and finish of each sampling run.

known since Darwin's time⁶. Biological material transported from the continents has been identified as discrete particles such as freshwater organisms, fungal hyphae, spores, plant fragments and insects^{3,4,6,7}. Terrigenous lipids such as the prevalent vascular plant waxes have been described as components of some aeolian dusts. Here we discuss the occurrence, isolation and characterisation of terrigenous lipids in aeolian dusts from the eastern Atlantic. Such lipids have also been found in aeolian dust from other oceanic areas⁸ and could be correlated with sedimentary lipids from deep-sea areas which have a large input of aeolian mineral components⁹. Lipids derived from terrigenous (higher plant) sources are primarily characterised by the distributions of the *n*-alkanes from *n*-C₂₅ to *n*-C₃₅ (odd carbon number predominance), the *n*-fatty acids from *n*-C₂₂ to *n*-C₃₂ (even carbon number predominance) and *n*-fatty alcohols from *n*-C₂₅ to *n*-C₃₂ (even carbon number predominance)⁹. Lipids derived from microbiota of either marine or lacustrine environments are characterised by the absence of these higher molecular weight homologues (> C₂₅) and the presence of *n*-alkanes ranging from *n*-C₁₃ to *n*-C₂₃ with a major maximum at C₁₇ and no significant carbon number predominance, and *n*-fatty acids ranging from *n*-C₁₂ to *n*-C₂₀ with a major maximum at C₁₆ and an even predominance⁹.

Collection and extraction of samples

Small dust samples were obtained from the research collection of the Department of Oceanography of the University of Liverpool. The samples had been stored in glass vials since completion of the inorganic analyses^{10,11}. The net-sampling procedure used here has been applied extensively for inorganic analyses of airborne particulates^{4,11,12}. The collection efficiency of the nets has been investigated in detail by various authors^{4,6,13} and it was found that such nets collect only about 50% of the < 2- μm size fraction and about 70% of the < 4- μm size fraction. The dust particles adhere to the net by concurrent wetting with sea spray and are washed salt-free afterwards⁹⁻¹².

The dust samples were extracted with two aliquots of toluene and methanol (3:1) for lipid analysis, and the extracts were concentrated on a rotary evaporator. After capillary gas chromatographic (GC) and gas chromatography-mass spectrometric (GC/MS) analyses all total extracts were treated with

diazomethane in ether and re-analysed by GC and GC/MS. Some extracts were further treated with *N,O*-bis-trimethyl silylacetamide (BSA) reagent to derivatise alcohols to trimethylsilyl (TMS) ethers and then subjected to GC and GC/MS analysis. The large sample extracts were separated by thin-layer chromatography (TLC) on silica gel with hexane-ether (9:1) as eluant into fractions corresponding to the retention times of hydrocarbons, esters and alcohols. These fractions were also subjected to instrumental analysis. The approximate yields of total lipids were estimated by weighing a 1-to-5- μl aliquot of total extract, after the solvent was allowed to evaporate, on a Cahn microbalance. These values were usually somewhat higher than those obtained by the sums of the integrations of the GC results from the hydrocarbon, acid ester and alcohol fractions. The instrumental analyses (GC and GC/MS) and the resultant detailed data have been described elsewhere^{8,9,14}.

The general collection data and descriptions for all samples are given in Table 1, and the sampling sites are shown on the map in Fig. 1. This sample suite represents a transect down the Atlantic Ocean from Spain past the tip (Cape of Good Hope) of South Africa (see Fig. 1). The beige Saharan dusts (samples 1 to 3) yielded relatively low amounts of solvent-soluble lipids. The dusts from the more tropical and the more vegetated environments (for example, samples 4 to 8) were grey to grey-brown and yielded larger amounts of solvent-soluble lipids.

The yields and analytical results for the *n*-alkanes and *n*-carboxylic acids are given in Table 2, together with some representative organic carbon values. The *n*-alkanes exhibited maxima at mainly *n*-C₂₅, *n*-C₃₁, and *n*-C₂₇, with strong odd-to-even carbon number predominances (see carbon preference index (CPI) in Table 2). The *n*-fatty acids maximised at *n*-C₁₆ and either *n*-C₂₄ or *n*-C₂₈, with strong even-to-odd carbon number predominances (see CPI in Table 2). The *n*-alkanes and *n*-fatty acids of all samples were distinguished from *iso*-, *anteiso*- and isoprenoidal compounds by coinjection of suitable standards on capillary GC, mass fragmentography and interpretation of individual low resolution mass spectra from GC/MS analyses. The *n*-alcohols exhibited a maximum at *n*-C₂₈, with a strong even-to-odd carbon number predominance (see CPI in Table 2). The distribution diagrams for all these lipid fractions can be found elsewhere¹⁴.

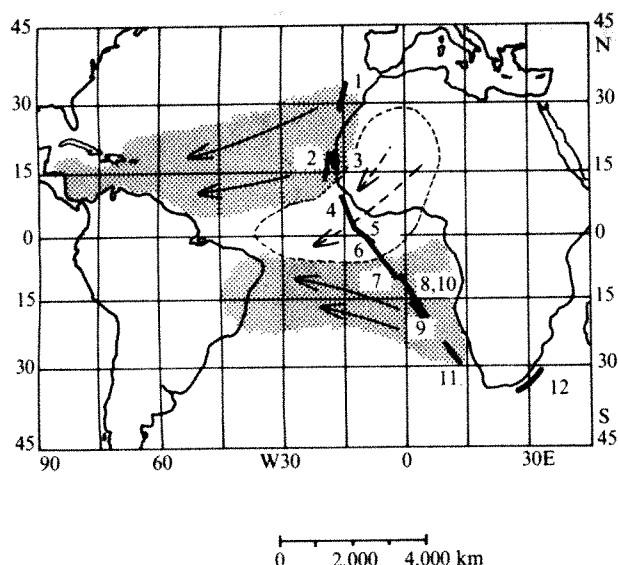


Fig. 1 Map showing the aeolian dust sampling locations and prevalent wind patterns (thick line, ship's track during aeolian dust collection; solid arrows, approximate extent of trade winds; dashed arrows, Harmattan wind).

Origin of samples

Sample 1 was taken off the coast of Morocco in the westerlies/north-east trades boundary zone and probably contains material derived from a variety of sources, including western Europe (for example, Spain) and the North African deserts. The extracted mixture consisted mainly of free fatty acids ranging from $n\text{-C}_{14}$ to $n\text{-C}_{20}$, with a maximum at $n\text{-C}_{16}$ and predominantly even homologues, and a minor amount of $n\text{-C}_{25}$ to $n\text{-C}_{33}$ alkanes. This acid distribution is a typical lacustrine and/or marine pattern⁹. Samples 2 and 3 consist mainly of the trade wind flux of Saharan material. The lipid yields were different for the two samples, but the distributions of homologues were essentially identical.

Samples 4 and 5 were collected off West Africa and probably represent mainly Saharan and West African material carried by the Harmattan winds (see Fig. 1)¹¹. Both lipid extracts exhibited distributions of homologues of predominantly $n\text{-C}_{25}$ to $n\text{-C}_{33}$ alkanes and $n\text{-C}_{22}$ to $n\text{-C}_{30}$ fatty acids, which are typical of higher plant wax¹⁵ and minor amounts of $n\text{-C}_{12}$ to $n\text{-C}_{20}$ fatty acids attributable to either a lacustrine or marine origin^{9,16}.

The remaining samples (6 to 10 and 12) represent fallout from the south-east trades (see Fig. 1), deriving material

mainly from the soils of southern Africa, the Namib and Kalahari deserts, and probably from the Lower Guinea area of Africa. Samples 9 and 10 were taken in the same area but at different seasonal times. The distributions of lipid homologues for these two samples were similar and the distributions of homologues for all the samples consisted of predominantly $n\text{-C}_{25}$ to $n\text{-C}_{33}$ alkanes and $n\text{-C}_{22}$ to $n\text{-C}_{30}$ fatty acids, and lesser amounts of $n\text{-C}_{12}$ to $n\text{-C}_{20}$ fatty acids.

The extracts of several samples (1, 6, 8 and 9) contained mono-unsaturated fatty acids (mainly $\text{C}_{18:1}$ and some $\text{C}_{16:1}$) in significant amounts. Trace amounts of triterpenoidal compounds were detected in the samples with a high plant wax component (such as 4, 5, 6, and 8) but not in the samples derived from more arid terrain (such as 1 to 3). Diterpenoidal compounds which have been proposed as terrigenous markers¹⁷ were not detected in any aeolian dust extracts examined thus far. Polynuclear aromatic hydrocarbons have also not been detected as any significant components (GC/MS detection limit approximately 0.1 p.p.m. per component). The problems of plasticiser and shipboard contamination were not serious and have been described in detail^{8,14,18}. Sample 11 represents an example of shipboard contamination where the lipids exhibited a general 'hump' distribution, typical of petroliferous material.

The n -alkanes, n -alkanoic acids and n -alcohols in the lipids of these aeolian dusts correlate well with terrigenous sources.

The n -alkanes observed in these dusts have essentially no homologues lower than about C_{22} and their distribution patterns corroborate a terrigenous origin from higher plant waxes^{15,19-21}. The extreme fragility of the plant wax protrusions has been described (ref. 22 and references cited therein). Wax particles are easily sloughed off from plant surfaces and can become airborne. There are not enough data points at this time to ascribe a significance or specific plant sources to the shifts of the maxima in the n -alkane distributions from $n\text{-C}_{27}$ in the North Atlantic (1), to $n\text{-C}_{27} \cong n\text{-C}_{29}$ (2, 3), to $n\text{-C}_{29}$ in the Inter-Tropical Convergence Zone (4 to 6), to $n\text{-C}_{29} \cong n\text{-C}_{31}$ (8), and to $n\text{-C}_{31}$ in the South Atlantic (9, 10) (see Table 2).

The n -alkanoic acid distributions of all the dusts exhibit a typically lacustrine and/or marine pattern, with maxima at C_{16} and superimposed on that a higher plant distribution pattern. Such fatty acid distributions have been described by many authors for sediments (see for example, ref. 23). This observation fits in part with Kolbe's proposal¹⁶ that aeolian dusts have a component derived from dessicated lacustrine muds. However, since the sampling nets were wet with sea-spray, some of the $n\text{-C}_{12}$ to $n\text{-C}_{20}$ fatty acids may also have a marine origin. Normal carboxylic acids with maxima at C_{24} or C_{26} and a strong even-to-odd predominance have been used as terrigenous

Table 2 Analytical results for aeolian dusts

Sample	Organic carbon (%) [*]	Total lipids		n -Alkanes			n -Fatty acids			n -Alcohols		
		(μg per g of dust)	(ng per m^3 of air)	Approx. yield ($\mu\text{g g}^{-1}$)	CPI [†]	Max. at C no. [‡]	Approx. yield ($\mu\text{g g}^{-1}$)	CPI [†]	Max. at C no. [‡]	Approx. yield ($\mu\text{g g}^{-1}$)	CPI [†]	Max. at C no.
1	12	750	0.13	10	10.2	27	740	n.d.	16	n.d.	—	—
2	2	130	4.7	40	1.4	25, 29	20	6.1	16, 24	70	4.6	28
3	2	460	10.0	400	7.5	27 $\cong$ 29	35	22	16, 24	n.d.	—	—
4	7	850	8.0	340	5.0	29	160	11.6	16, 20	240	2.5	28
5	12	2,400	1.0	860	3.2	29	900	7.5	16, 24	n.d.	—	—
6	10	9,800	1.8	2,200	5.6	29	3,400	14	16, 24	2,600	4.2	28
7	8	370	0.03	45	6.0	29 $\cong$	n.d.	—	—	n.d.	—	—
8	8	2,700	3.8	1,150	5.2	29 $\cong$ 31	400	4.1	26	650	3.3	28
9	8	270	0.08	120	6.5	29 $\cong$ 31	40	26	16, 26	n.d.	—	—
10	8	100	0.2	40	5.2	31	12	7.5	16, 26	n.d.	—	—
11	80	n.d.	n.d.	n.d.	—	hump	n.d.	—	—	n.d.	—	—
12	9	3,050	12.0	1,050	7.5	27	260	8.4	16, 26	n.d.	—	—

^{*}Ref. 12 (values for samples taken in the same area).

[†]Carbon preference index (CPI)^{33,34} was calculated for all homologues from C_{10} to C_{35} .

[‡]The major distribution maximum is underscored.

n.d., Not determined.

markers²⁴⁻²⁶ and these homologues from the dusts thus probably also represent a higher plant wax component. A shift of the maxima in the *n*-fatty acid distributions, analogous to the *n*-alkanes, was observed from *n*-C₂₄ in the North Atlantic samples to *n*-C₂₆ in the Mid- to South Atlantic samples. Again, no specific plant sources can be assigned to these limited data.

The higher molecular weight *n*-alcohol distributions observed for the dust samples (see Table 2) closely resemble the distributions of alcohols from plant wax. These consist of predominantly even-carbon-numbered homologues from *n*-C₁₆ to *n*-C₃₀, usually with a maximum at *n*-C₂₈^{15,21}. The presence of traces of triterpenoids in these dusts indicates that these compounds can be derived from a terrigenous source. The amounts of mono-unsaturated fatty acids (*n*-C_{16:1} and *n*-C_{18:1}) when considered with the distribution patterns of the saturated *n*-fatty acids < C₂₀, indicate a lacustrine and/or marine origin for these components. The presence of these labile compounds in the dusts also indicates that the destruction (photo-oxidation) of organic matter by ultraviolet radiation during transit is limited. The variations in the homologue distributions of the dust lipids from the relatively close geographical areas (for example, 9 and 10, 2 and 3, 5 and 6) may probably be accounted for by seasonal variations of plant source material and wind pattern change.

Anthropogenic components were found in dusts derived from urban and industrialised areas⁸. The hydrocarbons of aeolian dusts collected near urban centres have quite a different compound distribution, attributable mainly to petroleum product spillage and combustion (see for example refs 27, 28). The *n*-alkanes of urban air particulates are present as minor components as part of a general 'hump' typical of petroleum and they exhibit no carbon number predominance. On the other hand, the *n*-alkanes of the oceanic dusts are present as the major components and exhibit a very strong odd-to-even carbon number predominance. The sampling procedure using nets (meshes), however, does not collect aerosol particles, which can be major components of urban atmospheres (ref. 29 for example). Some oceanic dusts collected in proximities of urbanised areas (for example, inland sea off Japan⁸) also exhibit a 'hump' in the hydrocarbon fraction.

The sedimentation rates in the North Atlantic Ocean for the aeolian components off West Africa have been estimated to be about 0.6 mm per 1,000 yr (ref. 4). This figure is in good agreement with those reported by Goldberg and Griffin³⁰ for the equatorial regions of the Atlantic. Observed sedimentation rates adjacent to the Canary and Cape Verde Islands, based on ¹⁴C dates, range from 2-3 cm per 1,000 yr (ref. 31). A more recent input estimate of organic matter from dust to the ocean has been proposed³². It was found that during North African dust storms, extremely large amounts (> 10,000 µg m⁻³ at the coast) of dust are transported out to sea along about a 100 km stretch of coastline, thus also injecting a large organic component (about 20,000 tonnes of organic carbon per storm). Thus aeolian influx to the marine environment occurs in random major events and continuously in lower amounts.

The bulk of the lipid matter of aeolian dusts represents higher plant wax¹⁵ and fatty acids from lacustrine microbiota^{4,16} or possibly from sea-spray. It should be noted that the lipid yields for these samples are low when compared to the organic carbon contents of other samples from similar areas (see Table 2). No total carbon values could be determined for this sample suite. This difference may be partially due to a particulate carbon component from ships and a loss of volatile lipids during the analysis. Higher lipid yields against organic carbon were obtained for samples collected at the coastline of West Africa³². Work is proceeding to understand the budget of the influx of aeolian organic carbon compounds to the oceanic environment. It is, however, apparent that the input of aeolian material to certain sedimentary areas can be significant over geological time periods.

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letters to nature

Upper limits to X-ray emission from nova Vulpeculae 1976

Two recent optical novae have been observed to radiate a substantial flux of medium energy X rays (nova Monocerotis¹, and A1524-61 (ref. 2)), the X-ray luminosity in both cases reached a maximum at the same time as the optical

luminosity and was over 100 times greater. In contrast, an unsuccessful search was made by Hoffman *et al.*³ for X-ray emission from nova Cygni 1975 and the ratio of X-ray to optical luminosity in that case was found to be less than 10⁻⁴. Brecher and Morrison⁴ have suggested that the large differences in this ratio might be related to the rate of mass exchange in the binary system, believing that the X-ray

Table 1 X-ray upper limits

Mid-time (1976)	Orbit number	X-ray flux, L_x ($\text{erg cm}^{-2} \text{s}^{-1}$)
Oct 20.86	11184	$< 5.5 \times 10^{-9}$
20.92	11185	$< 3.7 \times 10^{-9}$
20.99	11186	$< 3.3 \times 10^{-9}$
21.07	11187	$< 3.4 \times 10^{-9}$
21.13	11189	$< 3.5 \times 10^{-9}$
21.20	11190	$< 3.5 \times 10^{-9}$
21.27	11191	$< 3.3 \times 10^{-9}$
21.33	11192	$< 3.2 \times 10^{-9}$
21.39	11193	$< 3.2 \times 10^{-9}$
21.53	11194	$< 3.2 \times 10^{-9}$

emission arises from the shock heating of dense circumstellar material by the nova ejecta. Only systems with a high density in the accretion flow, such as recurrent novae, will therefore generate large X-ray luminosities.

There is little observational evidence to test a relationship between recurrent activity and X-ray emission in optical novae; consequently, a search has been made through Ariel V data for times at which an optical nova was within the 17° field of view of the Rotation Modulation Collimator (Experiment A). Ten orbits of observations in October 1976 coincided with the maximum of nova Vulpeculae which was within the field of view from October 20.83 UT to October 21.53 UT. The optical outburst of this nova was detected by Alcock⁵ at a visual magnitude of 6.5 on October 21.764 UT. Gorynya⁶ reported a photographic magnitude of 8.8 on 20.696 UT which, together with a pre-outburst measurement by Shao⁷ of $m_{\text{phot}}=18.3$, indicated that the nova was near maximum during the Ariel V observations, and hence a likely source of X-ray emission.

No significant X-ray flux was detected and so an upper limit to the ratio of X-ray to optical luminosity of $\sim 10^{-1}$ can be set from these measurements, suggesting that nova Vulpeculae 1976 may be similar to nova Cygni 1975 in this respect.

Table 1 lists the upper limits to the X-ray flux from nova Vulpeculae for each orbit, together with the mid-time of each data integration. The upper limits are uncertain to within a factor of two due to the non-uniform spectral response of the detectors which are sensitive in the energy range 2.94–7.60 keV.

Table 2 is a compilation of the measurements of X-ray emission from optical novae and contains the ratio of X-ray to optical luminosity and the available evidence for recurrent

Table 2 X-ray emission from optical novae

Name	L_x/L_{opt}	Evidence for previous optical activity
A1524-61	$\sim 1.5 \times 10^2$	None reported, probably too faint for plate searches
nova Monocerotis 1975	$\sim 10^3$	Previous outburst, reported by Eachus ⁸
nova Cygni 1975	$< 10^{-4}$	No search reported
nova Vulpeculae 1976	$< 10^{-1}$	No previous activity back to 1896 in Harvard plate collection

optical activity. A search has been made by L. J. Chaisson (personal communication) in the Harvard Plate Collection for previous outbursts from nova Vulpeculae but none have been detected back to 1896, consequently the small amount of data available at present in Table 2 does not conflict with the hypothesis that X-ray emission accompanies recurrent activity in optical novae. The study of optical novae would clearly benefit from more X-ray observations at the period of maximum light.

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Electron-positron flux at the surface of the pulsar in the Crab

LARGE magnetic fields at the surface of neutron stars favour the formation of a condensed phase of electrons and positrons. It is possible to account for the narrow γ -ray line recently observed in the Crab spectrum from this phase with a flux of electrons and positrons at the surface of the neutron star in the Crab consistent with a recent model.

Leventhal, MacCallum and Watts¹ have, with a balloon-borne γ -ray telescope, recently detected a line feature at 400 keV emanating from the Crab nebula. This energy has the correct magnitude of red shift to be due to two-photon electron-positron (e^-e^+) annihilations at the surface of the pulsar in the Crab. About 10^{-3} photons $\text{cm}^{-2} \text{s}^{-1}$ are detected, which if they are emitted isotropically imply that the rate of annihilations at the pulsar is at least 10^{41}s^{-1} . If the annihilations take place in the polar gap region alone, and the photons travel along the magnetic field lines, this number is reduced to about 10^{40}s^{-1} . The most surprising feature of Leventhal *et al.* observations is the width of the line which is determined by the resolution of the detector to be less than 3 keV. One expects the spectrum over which the annihilation photons are distributed to be determined by the transverse momentum of the electrons and positrons in the magnetic field. For a field of 10^{12}G , this may be determined to be about 50 keV. Fields less than 10^{12}G are considered unacceptable at the surface of the pulsar in the Crab.

A possible mechanism to have narrow lines from e^-e^+ annihilation is for the annihilation to be stimulated by annihilation photons. This leads to the amplification of photon density near a particular energy. Stimulated annihilation processes require population inversion. In the present context this means that the electron and positron fluids be nearly degenerate—the density be such that the Fermi energy is much higher than the temperature.

The large magnetic field at the surface of neutron stars favours the condensation of electrons and positrons into rod-shaped droplets. The density in the droplets is high enough to obtain degeneracy.

Following Ruderman's² calculations of the properties of matter in superstrong magnetic fields, Chui³ has variationally estimated the binding energy of an electron-hole droplet in large magnetic fields in a semiconductor. One can equally well use this estimate for an e^-e^+ fluid. The variational estimate of the binding energy is

$$E_b = -13.4 (a_0/\rho_0)^{2/3} e^2/a_0 \quad (1)$$

where a_0 is the Bohr radius and ρ_0 is the cyclotron radius. The

radial extent of the electron or positron wave function transverse to the field

$$r \simeq 1.1 a_0 (\rho_0/a_0)^{2/3} \quad (2)$$

the linear density of the drop along the magnetic field

$$l^{-1} \simeq 1.72 a_0^{-1} (a_0/\rho_0)^{2/3} \quad (3)$$

and the Fermi energy of the one-dimensional fluid is

$$E_F = (h^2/2m)l^{-1} \quad (4)$$

For $B = 10^{12}$ G, one gets $\rho_0 \simeq 4.9 \times 10^{-3} a_0$, $r \simeq 0.15 a_0$, $l \simeq 0.075 a_0$, $E_b \simeq -2.75$ keV, and $E_F \simeq 3.9$ keV. Since the temperature at the surface of the neutron star in the Crab is estimated to be less than 0.5 keV, I concluded that if a steady state with the above parameters is attained, the droplet is indeed degenerate.

I shall next consider whether a steady state can be reached. The electrons and positrons arrange themselves in parallel rods among which there will be a weak residual van der Waal's attraction which may or may not be sufficient to bind them to each other. If they bind, the rods will be spaced at several times $2r_0$ in a droplet; say equal to αr_0 . In each rod, the spontaneous annihilation rate for e^-e^+ pairs is approximately given by

$$\tau_s^{-1} \simeq r_0^2 \rho v \quad (5)$$

where r_0 is the classical electron radius, v is the Fermi velocity and ρ the density. The total number of annihilations per second per unit volume is $r_0^2 \rho^2 v$. For the parameters listed above one estimates this to be $10^{38} \text{ s}^{-1} \text{ cm}^{-3}$.

Suppose there is a flux density of e^-e^+ of $\phi \text{ cm}^{-2} \text{ s}^{-1}$ in a given region at the surface of a pulsar. One can roughly estimate the steady-state radius of the droplets, R by equating the annihilations with the introduction of pairs into the droplets assuming the latter occur uniformly over the surface of the droplet. If $R \gg r$, one may conclude that a steady state is possible, and not otherwise. I estimated that $R \simeq 10^{-38} \phi \alpha^2 \text{ cm}$. For the worst case, $\alpha = 1$, one requires $\phi \gg \phi_0 \simeq 10^{38} \text{ cm}^{-2} \text{ s}^{-1}$ to get $R \gg r$.

In one of the more successful models for radiation mechanisms from pulsars^{4,5}, e^-e^+ positron discharges have a crucial role. A flux density of only 10^{24} s^{-1} is allowed in these models from discharges at the 'near gap'⁵. However, an 'outer gap' has been proposed⁶ which generates photons of high enough energy that pair production on magnetic field lines ensues. A flux $\phi \gg \phi_0$ at the surface of the neutron star in the Crab in a region of the polar cap outside the projection of the 'near gap' is quite consistent with this model. Thus the existence of the e^-e^+ droplets depends on this model. Such a flux also accounts for the total number of e^-e^+ annihilations observed by Leventhal *et al.*¹.

Having examined the conditions and characteristics of stimulated annihilation radiation from the droplets, I found that for the length of the droplet greater than 10^{-4} cm , nonlinear amplification of the photons travelling along the length of the drop occurs. The amplification rate is time dependent and the linewidth depends on the amplification rate and can on the average be much less than 3 keV. I also found that the photons thus emitted can escape the magnetosphere around the pulsar with negligible loss. I will publish the details of these calculations later.

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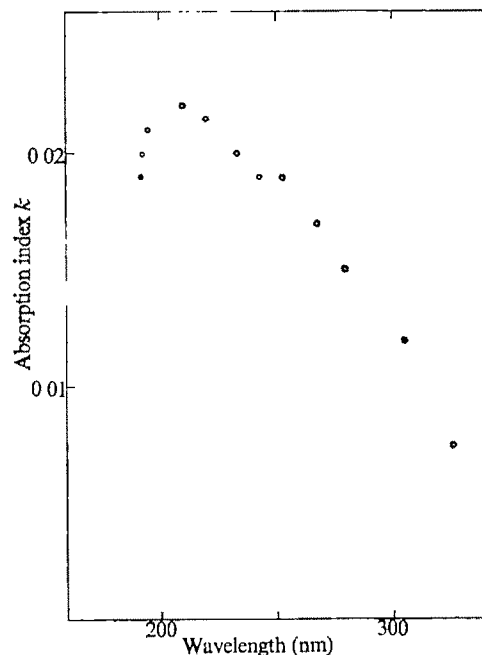
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Pre-biotic molecules in Martian dust clouds

THE search for life on Mars has so far proved disappointing¹. Experiments on the Viking package were designed on the presumption that any microbial life on Mars would be endowed with metabolic processes similar to present-day microorganisms on Earth. Analyses of gases released in soil heating experiments² have provided no evidence for significant abundances of highly complex organic molecules. These data are however, limited to only two landing sites and moreover the compositional studies carried out so far reflect properties of only a relatively thin dust layer on the Martian surface. Dust sucked up into the atmosphere from large areas of the planet's surface would have been witnessed in the 1971 storm, representing a much broader sampling of Martian surface material. Here we propose that a broad absorption feature centred on a wavelength close to 2,200 Å, derived from scattering studies of these dust clouds, may be a signature of highly complex organic or prebiotic molecules.

Recently, the possibility that a primitive life form could evolve in the interstellar medium resulting from the formation and destruction of clumps of inorganic grains coated with organic polymers has been discussed³. The identification of the well-known 2,200-Å interstellar absorption band with electronic transitions in a wide class of organic molecules provides tentative support for this

Fig. 1 Absorption index k of Martian dust (1971 dust storms) from Pang and Ajello⁴.



proposal—at any rate for the occurrence of highly complex organic molecules within grains. Also, a similar feature found by Sakata *et al.*⁴ in the organic matter extracted from a carbonaceous chondrite is suggestive of a dormant proto life form in the nature of interstellar grain clumps.

Pang and Ajello⁵ have recently deduced the wavelength dependence of the absorption index k (complex refractive index $m = n - ik$) of Martian dust from an analysis of the scattered light in the 1971 dust clouds observed by Mariner 9. Such clouds are swept up from a large area of the Martian surface and their composition may thus be more representative than that of the exceedingly small sample studied in the Viking experiments. The absorption index deduced from these data is negligible throughout visible wavelengths but increases in the ultraviolet to show a conspicuous hump at a wavelength close to $\lambda = 2,200 \text{ \AA}$ (Fig. 1). The half-width of this band, $\sim 150 \text{ \AA}$, is similar to that of the interstellar extinction band, and also to the absorption band associated with complex organic molecules of the type discussed by Hoyle and Wickramasinghe³. Although a tentative identification of this band with TiO_2 (anastase) grains has been suggested³, an association with pre-biotic organic molecules securely lodged within clumps of refractory grains is a distinct possibility which cannot be ruled out by the present data. We note that the strength of the interstellar $\lambda = 2,200 \text{ \AA}$ band is incompatible with absorption by TiO_2 grains (assuming solar abundances), whereas $\sim 10\%$ of interstellar C, N, O in the form of pre-biotic molecules may be shown to provide the observed band strength. Similarly, there would seem to be no compelling geological reason for an over-abundance of Ti on the Martian surface. On the contrary, studies of Martian soil have given an upper limit of 1% (ref. 6) by mass to the titanium content, whereas the titanium composition of dust in the 1971 cloud required to explain the data in Fig. 1 is a few percent³. More accurate and extensive spectroscopic studies of Martian dust clouds may well hold vital clues which have a bearing on the question of complex organic molecules and primitive life forms on Mars, as well as their possible connection with a meteoritic and interstellar origin.

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Jurassic sea-floor spreading in the eastern Equatorial Atlantic

THE history of sea-floor spreading in the Equatorial Atlantic is poorly known, having been largely inferred from the configuration of fracture zones and the geometry of magnetic lineations in other parts of the Atlantic (Fig. 1). Uncertainties in determining crustal ages at these low latitudes arise partly because of the small amplitudes of the magnetic anomalies and partly because of structural complexities, the separation of South America and Africa involving movements along closely spaced, east-west fracture zones¹⁻³. In this report we describe a series of magnetic anomalies in the eastern Equatorial Atlantic which can be dated using the Vine-Matthews hypothesis⁴ and the time scale of geomagnetic reversals derived by

Larson and Hilde⁵. The age of the anomalies provides an important constraint on the timing of the early rifting phase of the Atlantic off Guinea, Sierra Leone and Liberia.

Three magnetic profiles recorded from RRS Shackleton in 1974-75 are presented in Fig. 2. Also included is a published set of anomalies⁶ observed along a WNW-ESE track at 20°N and a corresponding profile computed for a simple spreading model. At 20°N Hayes and Rabinowitz⁶ identified anomalies M2 (Barremian) to M25 (Oxfordian) and have extended this sequence south of the Cape Verde Islands to the vicinity of a fracture zone at 13°N . A magnetically quiet zone occurs to the east which, in its outer portion at least, seems to have resulted from sea-floor spreading during a long period of constant magnetic polarity that ended in the Lower Oxfordian^{5,6} (Fig. 2). Profile 1 lies 110 km south of Glomar Challenger site 367 where sediments of Oxfordian(?)–Kimmeridgian age have been found resting on basaltic basement⁷. On this line, Upper Jurassic anomalies M19–M24 can be identified, together with anomalies of Lower Cretaceous age. The pattern is displaced 160 km in the left-lateral sense from the lineations south-east of the Cape Verde Islands. Whether the displacement takes place entirely at the 13°N fracture zone or along a series of faults cannot be evaluated from present information. A magnetically quiet region occurs on profile 1 but it is significantly narrower than that observed further north (Fig. 1).

Anomalies along tracks between 11°N and 7°N cannot be correlated with those on profile 1, probably because of structural complications associated with the Guinea⁸ and Sierra Leone⁹ fracture zones. Mesozoic lineations are, however, again identified further south. The sequence is

Fig. 1 Mesozoic magnetic lineations in the Eastern Equatorial Atlantic, numbered according to the reversal time-scale of Larson and Hilde⁵. Anomalies north of 13°N are taken from Hayes and Rabinowitz⁶. The inset map is based on crustal ages given by Pitman and others²⁷ and bathymetry is drawn from charts of Uchupi²⁸. Lines 1–6 are tracks of RRS Shackleton. Magnetic anomalies along profiles 7 and 8 have been made available by the Woods Hole Oceanographic Institution and the United States Geological Survey, respectively. Positions 13 and 366–368 are sites drilled by Glomar Challenger. Ages of oldest sediments recovered are: 13 (Senonian; acoustic basement not reached²⁹), 366 (Maestrichtian; acoustic basement not penetrated⁷), 367 (? Oxfordian to Kimmeridgian; acoustic basement penetrated⁷), 368 (Albian; acoustic basement not reached⁷).

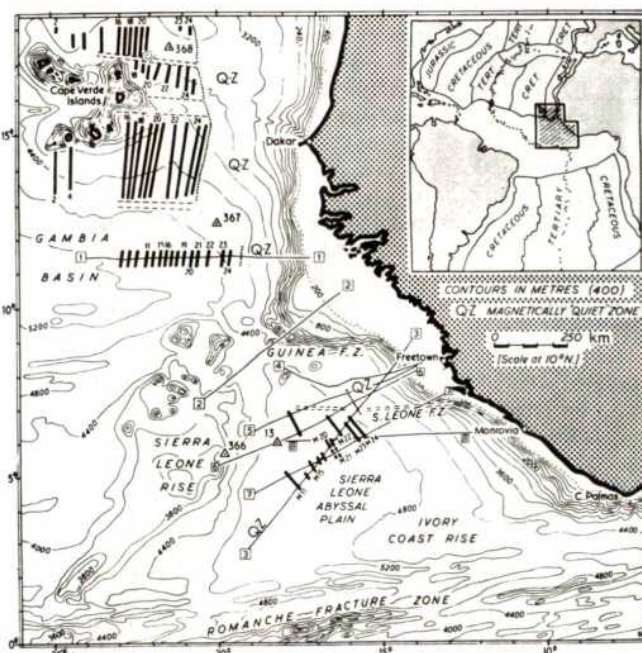
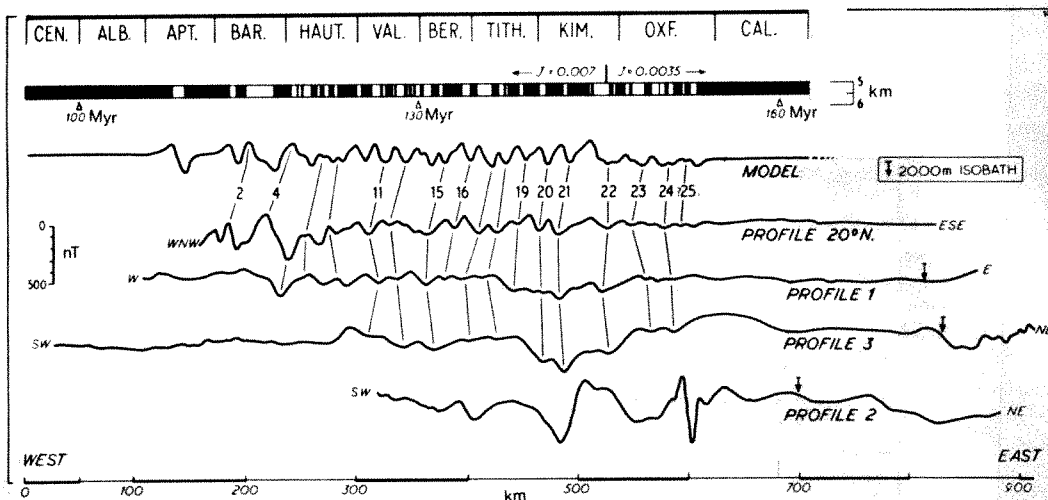


Fig. 2 Correlation of magnetic anomalies. Profiles marked Model and 20°N are taken from Hayes and Rabinowitz⁶. The former profile was calculated using the simple spreading model at the top of the figure. Shaded blocks are normally magnetised. A change in the magnetisation of the basement from 0.007 to 0.0035 electromagnetic units occurs at the position indicated. Anomalies are numbered according to the timescale of Larson and Hilde⁵. Note that a magnetically quiet region immediately west of the continental slope is absent on profile 2, which crosses the Guinea fracture zone.



most complete on profile 3, with the distinctive Kimmeridgian–Oxfordian anomalies M20–M24 clearly defined (Fig. 2). Although of small amplitude, M17 and M18 of Tithonian age can be recognised, in addition to Lower Cretaceous anomalies M11–M15. Correlation with adjacent tracks indicates that the anomalies trend in approximately the same direction as the Liberian continental slope and are displaced about 370 km to the east of those in the central Gambia Basin.

Crustal ages adjacent to the West African margin and in the south-western part of the Sierra Leone Basin are a matter of conjecture since the M-sequence on profile 3 is bounded by magnetically quiet regions, which are open to a number of interpretations^{10,11}. An indication of their nature in this region is provided by their relationship to crustal structure, shown in Fig. 3. The structural section is primarily based on published seismic refraction results¹², although additional gravity and seismic reflection profiles recorded from RRS Shackleton have been taken into account in computing the crustal model presented here. The abrupt transition from the M-sequence to the outer quiet zone coincides with the limit of an area of unusually thick crust, characterised by high seismic velocities (7.0–7.3 km s⁻¹) in its lower parts. The edge of the quiet region therefore seems to be a structural boundary. The composition of the 7.0–7.3 km s⁻¹ material cannot be interpreted unambiguously, but it is probable that it is largely made up of high-grade metamorphic equivalents of the gabbroic 6.2–6.7 km s⁻¹ layer¹². Thus, one explanation of the magnetic smoothness is that it has arisen from a period of crustal reheating during which the basement magnetisation acquired at the ridge crest was destroyed, the crust later becoming remagnetised in a uniform direction. Such a metamorphic episode may have been closely related to the uplift of the Sierra Leone Rise (Fig. 1). The magnetically quiet zone is clearly not restricted, however, to the region with high basement velocities, as it continues for at least a further 90 km to the south-west (km 1,130–1,220, Fig. 3). The outer section of normal oceanic crust is extensive enough to suggest that it was formed during the long post-Aptian period of normal magnetic polarity (Fig. 2), a tentative interpretation which implies that the anomalous crust is no younger than mid-Cretaceous. A magnetic edge effect at the boundary near km 1,130 (Fig. 3) is absent, perhaps because the anomalous crust is magnetised in the same direction as the sea floor to the south-west. At these low latitudes, variations in the intensity of basement magnetisation, resulting from different cooling histories,

are unlikely to be discernible from surface magnetic profiles.

The M-sequence and most of the quiet zone lying adjacent to the continental margin occur in an area of normal crustal thickness for a deep ocean basin. The quiet region starts near the Sierra Leone fracture zone (F.Z., Fig. 3), which is recorded on seismic profiles as an area of complex basement topography, and ends where the Moho dips steeply eastwards, the termination being marked by a positive magnetic anomaly and steep free-air gravity gradient (near km 410, Fig. 3). A similar magnetic signature has been observed on other aseismic continental margins and can be attributed to an edge effect caused by the juxtaposition of weakly magnetised continental crust and strongly magnetic oceanic basement¹³. Its identification in this region and the presence of the Moho at normal oceanic depth under much of the quiet zone suggests that an extensive rift, floored by oceanic crust, was in existence off Sierra Leone and Liberia by Oxfordian (M24) time. The formation of the Sierra Leone Basin is thus related to the opening of the Atlantic further north and, therefore, to the movement of North America from Africa. During the early stages of rifting off Sierra Leone the South Atlantic remained closed, as the oldest oceanic magnetic anomalies south of the equator are of Lower Cretaceous (Valanginian) age¹⁴.

The development of an oceanic rift in the Sierra Leone Basin during the Jurassic appears to be broadly consistent with the history of adjacent parts of West Africa and offers a plausible explanation for intrusive activity and subsidence near the present continental margin during Jurassic–Cretaceous time. In Liberia and Sierra Leone the edge of the West African craton experienced an episode of igneous activity during the early Jurassic, with the intrusion of tholeiitic dykes parallel to the present continental margin and the emplacement of a deep-seated, basic complex around Freetown^{15–19}. The dykes are abundantly developed on land and are known to extend beneath the Liberian shelf, giving rise to linear magnetic anomalies¹⁶. The sharpness of the gravity high and the low amplitude, high frequency magnetic anomalies near km 340 in Fig. 3 probably reflect the continuation of the dyke swarm along the outer part of the Sierra Leone shelf. A tensional stress regime, symptomatic of the initial stages of Atlantic rifting, is thus indicated over a large part of this region during Jurassic time. It is interesting that basic dykes of early Jurassic age have also been reported on the eastern side of the Maranhão Basin in Northern Brazil^{20,21}. On the pre-drift

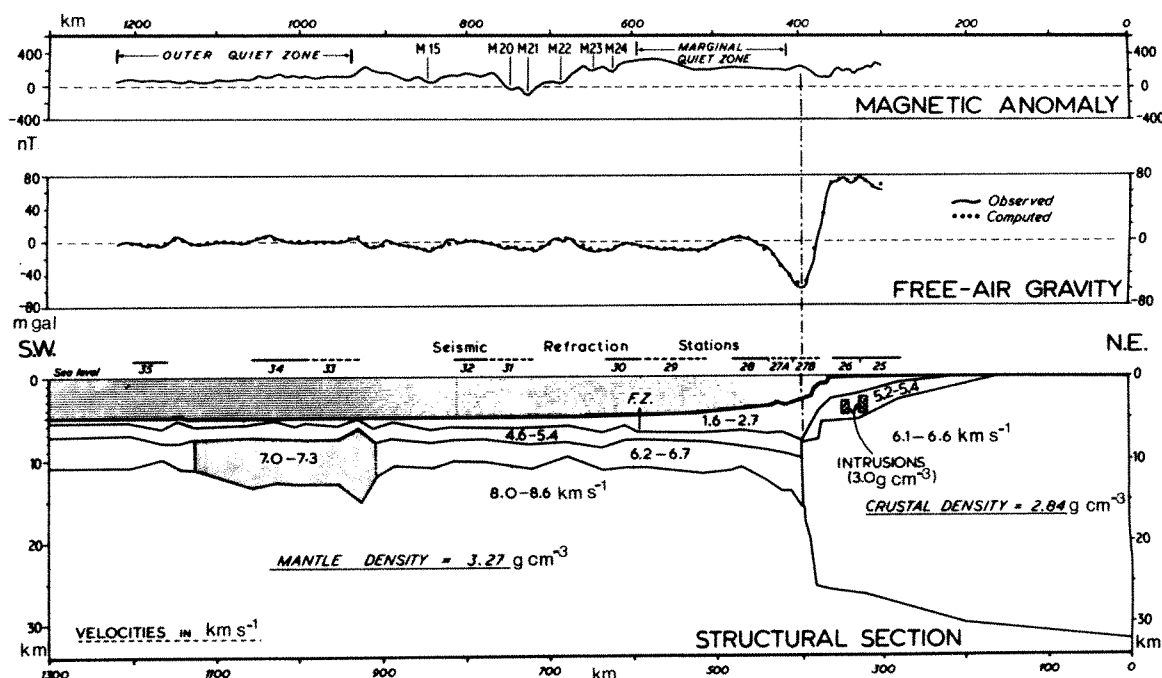


Fig. 3 Crustal structure along profile 3 in Fig. 1 based on published seismic refraction results¹² and the RRS Shackleton gravity profile. Seismic stations indicated by dotted lines are unreversed; the remainder are reversed profiles; F.Z. is the Sierra Leone fracture zone.

continental reconstruction of Bullard and others²² these intrusions form the southern continuation of the Liberian dyke swarm.

In the coastal region of Liberia the Jurassic igneous activity was followed in the Lower Cretaceous by a period of block faulting, subsidence and rapid sedimentation. Four offshore wells near Monrovia reveal a thick section (up to 1,940 m) of dominantly marine Lower Cretaceous sediments resting on basic volcanics, dated as Jurassic at one location and Lower Cretaceous at a second drilling site^{23,24}. Seismic data (ref. 12 and unpublished RRS Shackleton reflection profiles) suggest that more than 2 km of Mesozoic sediments, probably mostly Cretaceous in age, lie beneath the outer shelf off Sierra Leone. The Senegal margin is known to have subsided at least 4 km during the Cretaceous²⁵. However, in this area the first major Mesozoic transgression seems to have occurred before that in western Liberia, as thick marine limestones of Upper Jurassic age have been encountered in boreholes near Dakar. A somewhat later start to rifting off Sierra Leone and Liberia could account for the absence of a continuation of the Triassic-early Jurassic Senegal evaporites²⁶ south of the Guinea fracture zone.

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Late Quaternary lake levels in southern Afar and the adjacent Ethiopian Rift

A PERENNIAL problem in using evidence of changing lake levels and river activity to reconstruct Quaternary environments in East Africa stems from our frequent inability to distinguish between effects produced by volcanic and tectonic activity and those related to climatic change. We report here evidence for roughly synchronous changes in lake levels in the volcanically and tectonically active regions of southern Afar and the Ethiopian Rift, which parallel those reported from the Sahel¹, the Nile valley², Kenya³ and Saudi Arabia⁴. Such synchronous changes in lake levels,

allied to independent evidence of moister conditions during phases of high lake levels, are best explained through regional fluctuations in climate.

Lake Besaka is situated at the junction of the Ethiopian Rift and the Afar triangle (Fig. 1), and is bounded to the west and east by pre-Holocene fault scarps which run NNE-SSW, parallel to the regional trend of faults in the Wonji Fault Belt. The northern margin of the lake consists of very fresh, unvegetated aa lava which earlier observers^{5,6} attributed to an eruption in the 1820s. Morphologically fresh cinder cones mark the present southern edge of the lake (Fig. 2). Late Holocene to modern tensional fissures at least 30 m deep and up to 4 m wide extend north through the welded tuffs on the southern flanks of Fantale volcano.

The lithostratigraphic sequence above and below the western fault scarp at Lake Besaka (Fig. 3) shows that the lake attained a level of about 10 m above the January 1974 datum level towards 11,000–15,000 BP (Table 1), shortly after a major eruption of olive-green pumice sand and gravel. Above the scarp the pumice is an airfall deposit that covers a Late Stone Age site with nearly a metre of coarse sand and sporadic gravel. Overlying the green sand is a second Late Stone Age occupation layer with abundant obsidian implements, faunal remains and human burials. Quantitative analysis by X-ray fluorescence of the strontium, yttrium and zirconium content of the green sand above and below the scarp and within the marginal fissure site E3 W (Fig. 3) shows that the trace-element content is identical at all sites, as are the refractive indices of the glass and the mineral composition of the sand. The only differences lie in the sedimentary structures and upward coarsening evident in the green sand below the scarp, which reflect deposition within or resorting by the lake.

Fig. 1 Location map of sampled sites.

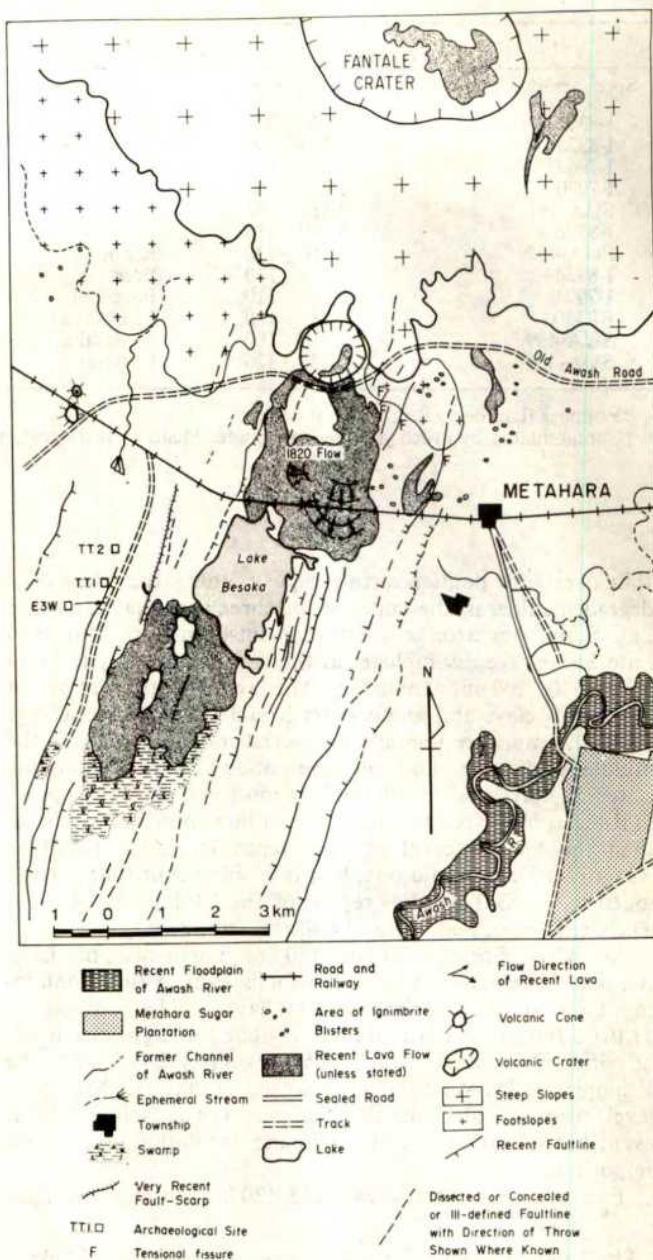
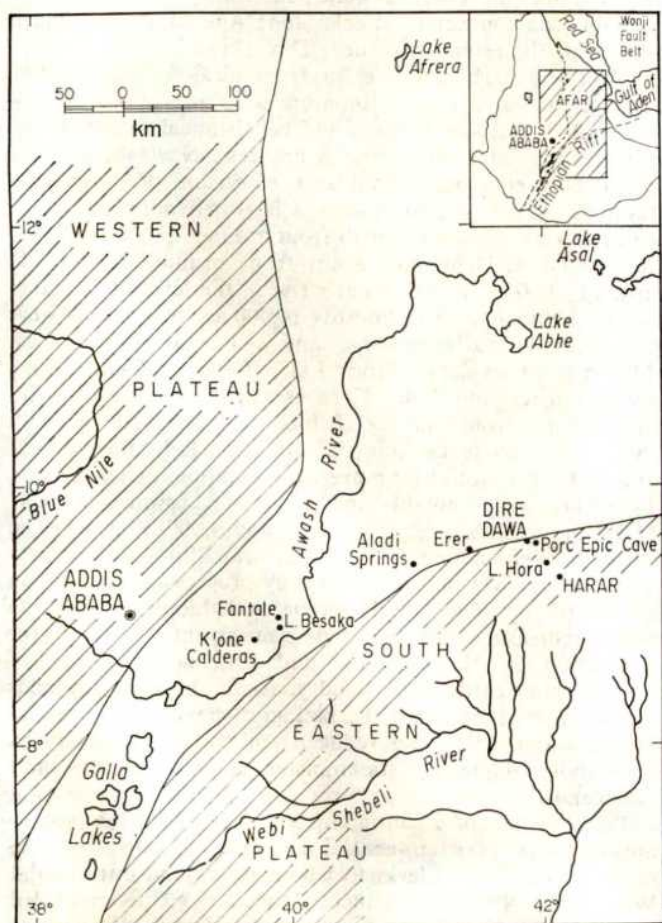


Fig. 2 Geomorphic map of Lake Besaka. The sketch map is based on air photographs taken in 1957.

The lake level was high ($\geq +10$ m) for an unknown interval during the late Pleistocene before the deposition of the green sand, and fell sufficiently for Late Stone Age man to move out across the former lake floor down to and perhaps lower than an elevation of +4.5 m.

A fall in level following deposition of the 11,000–11,500 BP shell-bearing diatomites above the green sand is evidenced by dark organic clays that locally contain Late Stone Age flakes. The lake rose again during the Holocene to reach the base of the fault scarp at +10 m, and a second layer of diatomite accumulated above the dark clay towards or shortly after 9,500 BP (Table 1). Before attempting to interpret the changing late Quaternary levels of Lake Besaka, it is pertinent to review evidence from the area as a whole (Fig. 1; Table 1).

K'one caldera complex⁷ lies in the Ethiopian Rift some 30 km WSW of Lake Besaka. Recent gully erosion has revealed a horizontal sequence of alternating dark grey clays and brown tuffaceous loams above the welded tuff and

Table 1 Radiocarbon ages from south-central Ethiopia

Specimen no.	^{14}C age (b.p.)	Site	Material	Comments
I-8322	$14,670 \pm 200$	K'one	Ostrich eggshell	Just below upper dark clay
I-8325	$11,430 \pm 380$	L. Besaka	Shells	<i>Melanoides</i> at 4.2 m
I-7969	$11,200 \pm 160$	L. Besaka	Shells	<i>Melanoides</i> at 6.5 m
I-7970	$11,070 \pm 160$	Aladi Springs	Shells	<i>Melanoides</i> in LSA tufa
SUA-461	$9,410 \pm 140$	L. Besaka	Shells	<i>Melanoides</i> at base of dark clay
I-8326	$6,890 \pm 115$	L. Hora	Shells	Mixed littoral assemblage
SUA-462	$6,810 \pm 120$	K'one	Carbonate*	Base of upper dark clay
I-8324	$6,670 \pm 110$	Erer	Shells	<i>Melanoides</i> in tufa
I-7971	$5,700 \pm 110$	Porc Epic	Charcoal*	In calcareous MSA breccia
I-8330	$4,785 \pm 120$	L. Besaka	Charcoal	Near top of LSA site
SUA-459	$4,750 \pm 160$	L. Besaka	Carbonate*†	LSA soil below green pumice
SUA-460	$4,220 \pm 120$	L. Besaka	Humic clay	Above shelly diatomite

*Younger than deposit in which it occurs.

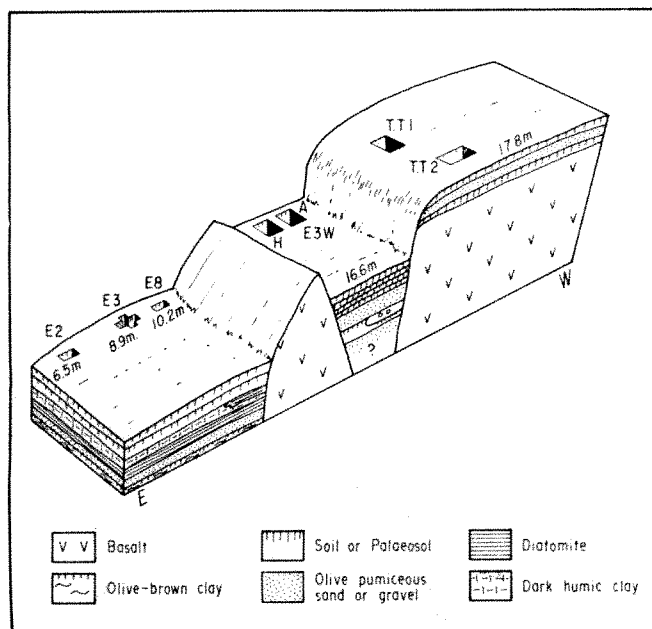
†Contaminated by much younger carbonate. Mean of two ages: inner sample: $4,490 \pm 115$; outer sample: $5,010 \pm 110$.

thin overlying pumice airfall deposit lining the floor of a degraded caldera. The upper of the three dark clays contains Late Stone Age artefacts, and the transition from Middle to Late Stone Age assemblages at the base of this clay is dated at $14,670 \pm 200$ BP (Table 1). The regular alternation of horizontal clays and loams from Middle to Late Stone Age time, the moisture-tolerant terrestrial mollusc fauna of the middle dark clay, and the present-day lack of standing water in this locality all imply deposition of the clays in moister conditions than those prevailing there today. If so, then the final interval of clay deposition after $14,670$ BP was a relatively humid one, and it is significant that further south in the Galla Lakes region of the Ethiopian Rift lake levels rose at or soon after $14,400$ BP (ref. 8).

At Aladi Springs, about 150 km north-east of Lake Besaka, *Melanoides tuberculata* shells from a tufa containing Late Stone Age implements have yielded an age of $11,070 \pm 160$ BP, which is similar to that for high lake levels at sites in central Afar⁹, the Awash valley¹⁰ and the Ethiopian Rift⁸. We conclude that the 11,000–11,500 BP high level recorded at Lake Besaka was not a solitary local event but was triggered by a climatic oscillation of regional importance.

Two southern Afar sites some 120 km apart show signs

Fig. 3 Block diagram of late Quaternary deposits west of Lake Besaka (not to scale).



of wetter conditions towards 7,000 BP. Lake Hora near Harar was at least 1 m deeper than now at $6,890 \pm 115$ BP; and a shelly tufa was deposited under permanent water on the flood-plain of a now seasonal stream between Aladi Springs and Erer at $6,670 \pm 110$ BP. Lakes Abhe, Afrera and Asal in central Afar⁹ were also very high at this time, and it may be no coincidence that calcium carbonate was being precipitated at the base of the upper dark clay at K'one some $6,810 \pm 120$ yr BP.

A Late Stone Age site above the western fault scarp at Lake Besaka is dated at $4,785 \pm 120$ BP for undisturbed occupation levels near the surface. Calcium carbonate nodules within the pre-Holocene lake clay beneath the green sand below the scarp have a mean age of $4,750 \pm 160$ BP, suggesting mobilisation of younger carbonate at about this time. A single anomalously young date of $5,700 \pm 110$ BP was obtained on charcoal collected from a late Pleistocene cave-breccia containing Middle Stone Age obsidian points at Porc Epic limestone cave near Dire Dawa.

Shell and carbonate are far from ideal for radiocarbon dating, so that a search for more suitable samples is now under way at Lake Besaka, and geochemical assays of the presently dated samples are in progress. Nevertheless, and despite the equivocal significance of certain of these dates (notably I-7971 and SUA-458) it is worth noting that the central Afar lakes⁹ were high from around 10,000–11,000 BP to around 8,500 BP and again from around 7,500 BP to around 4,000 BP. After an early rise in the level of the lakes of the Ethiopian Rift⁸ towards 14,400 BP, lake levels were very high just after 9,500 BP and again towards 6,500 BP. The present evidence from Lake Besaka, K'one, Aladi Springs, Erer and Lake Hora is entirely consistent with other data from outside Ethiopia^{1–4}, as well as with independent evidence from the Ethiopian Rift and Afar^{8–10}, and indicates wetter-than-present conditions after around 14,500 BP, with notably moist climates towards 11,000–11,500 BP, 9,500 BP, 6,500–7,000 BP and 4,000–5,000 BP. The early Holocene climates in south-central Ethiopia thus show signs of four wet pulses followed by progressive desiccation after 4,000 BP. The late Holocene in Ethiopia was a time when increasing burning and clearing of the land for cultivation in the uplands, and grazing by herds of domesticated cattle, sheep and goats in the drier lowlands would have accentuated the biological pressure exerted by falling water-tables and reduced soil-water content on a vegetation adapted to the moister climates of the early Holocene.

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The geochemical significance of positional isomers of unsaturated acids from an intertidal zone sediment

FATTY acids continue to provide insights into the origins and reactions of organic matter in Recent sediments^{1–4}. As part of a wider baseline study^{1,2} we have isolated the fatty acids from a typical temperate intertidal zone sediment located at the south-west of Corner Inlet, Victoria¹. The acid concentration in this core section is very low (13.4 µg per g sediment) and in contrast to most sediments the mono-unsaturated acids predominate over the saturated acids (Table 1). There are also significant quantities of polyunsaturated acids (Table 2) which have rarely been identified in sediments^{5,6} and there has been no detailed study of the isomers present. The carbon number pattern taken together with the degree of unsaturation and the isomer positions are used in this report to confirm a marine diatom input to the sediment distinguishable from that of other micro-organisms.

The sediment core (13–19 cm deep) was collected 6 m above the low water mark from beneath a bed of *Zostera marina* which stabilised the sediment. Terrestrial plant input is minimal. The sediment was sieved to remove any larger organisms and the total extract was obtained by

Table 1 Concentrations of different acid classes

Type	Number of double bonds	Carbon number (range)	Concentration (µg acid per 100 g sediment)
Straight chain	0	10–30	421.0
Branched*	0	11–19	91.0
Straight chain	1	12–18	517.0
<i>cis</i>			
Straight chain	1	12–18	13.3
<i>trans</i> †			
Branched	1	15–18	2.8
<i>cis</i>			
Straight chain	2	16–18	50.2
<i>cis</i>			
	3	16–20	49.3
	4	18–22	115.0
	5	20–22	82.9

* Mainly iso and anteiso, some cyclopropyl 17:0, 19:0, trace 10-methyl 16:0. † Only t-16:1, t-18:1 positively identified and quantitated.

KOH-methanol extraction¹. Rigorous precautions were taken to exclude contamination and blank runs were performed. The carboxylic acids were obtained by partitioning the acidified extract into heptane-diethyl ether mixtures. The methyl esters (BF₃–MeOH) were purified on silica gel and 5% AgNO₃ silica gel plates. Small quantities of long chain α,ω-dicarboxylic acids and hydroxylated acids were also present¹.

Gas-liquid chromatography utilised glass SCOT SE30 (60 m × 0.5 mm ID; S.G.E. Australia), and stainless steel capillary BDS (46.7 m × 0.25 mm ID; Perkin Elmer) columns. Compound identification was by co-injection and relative retention time measurements. Hydrogenation in methanol over Adams catalyst confirmed the presence of unsaturated acids.

The sedimentary fatty acid concentrations are high in 16:0 (18%), 16:1ω7, 20:4ω6 and 20:5ω3 and low in C₁₈ components (Table 2). This pattern closely resembles that of many species of marine diatoms^{7–9} and is quite unlike most other algal species^{10,11}. A diatom input is further suggested by the positional isomers of the polyunsaturated acids present.

The three 16:2 isomers commonly found in marine phytoplankton are ω4, ω6 and ω7 but only the isomers (ω4 and ω7) commonly found in marine diatoms^{9,10} are found in the sediment. Jamieson and Reid¹¹ reported only the ω4 and ω6 isomers in their extensive study of macroscopic Rhodophyta, Phaeophyta and Chlorophyta species. Similarly, of the three 16:3 isomers present, only the ω4 is in significant quantity. This is the only isomer reported by Ackman and Tocher¹⁰ for four different diatom species. The virtual absence of the ω3 isomer indicates that Chlorophyceae in particular can make only a small contribution to the sediment.

The low levels of C₁₈ unsaturates also suggest a minor contribution from higher plants or green algae. Of the two 18:3 isomers identified, it is the less abundant ω3 isomer which is common to plants and most algae^{10,11}. The tetra- and penta-unsaturated components isolated, while typical of marine diatoms^{7–10}, are also found in varying amounts in other algae¹¹. The complete absence of 22:6 reflects the small contribution from higher marine organisms.

The abundance of these highly unsaturated molecules in the sediment is unusual as it is generally believed that they are rapidly degraded in a sedimentary environment⁴. Cranwell¹² was unable to detect polyunsaturated acids in a lake sediment despite the fact that the presumed major source of the sediment fatty acids were planktonic organisms which contain large quantities of these acids.

This rapid degradation reflects the aerobic conditions to which the unsaturated acids presumably were exposed¹². In our sediment most of the unsaturated acids will be deposited directly into the sediment when the diatoms die, and hence the acids will largely escape the aerobic conditions at the surface. Preservation is then aided by the anaerobic conditions below the surface ($E_H \approx -120$ mV at the studied depth).

Bacteria are known to contribute branched (iso, anteiso and cyclopropyl) acids to sediments^{2,3} but their contribution to unsaturated acids has been less certain². Our sediment contains a wide range of mono-unsaturated acids and particularly significant levels of vaccenic acid (18:1ω7). Unlike plants and most algae^{9,10} bacteria usually have vaccenic acid and not oleic acid as the major 18:1 isomer¹³. We believe that bacteria contribute most of the 18:1ω7 and a significant proportion of the other mono-unsaturated acids in the sediment. Recent results (to be published) from bacteria isolated from the sediment support this view. Probably the branched unsaturated acids isolated are also derived from bacteria.

Van Vleet and Quinn¹⁴ have recently examined the 16:1 and 18:1 isomers in an estuarine sediment and found 8%

Table 2 Concentration of unsaturated acids

Acid	Percentage of total acids*	Concentration ($\mu\text{g per 100g sediment}$)	Acid	Percentage of total acids	Concentration ($\mu\text{g per 100g sediment}$)
12:1†	0.12	1.55	17:2†	0.28	3.64
14:1‡	0.24	3.26	18:2‡	1.34	18.0
14:1‡	0.12	1.54	16:3‡§	1.28	17.1
15:1‡	0.30	3.97	18:3‡	0.45	6.03
15:1‡	0.42	5.56	18:3‡	0.86	11.6
iso 15:1	0.11	1.51	20:3‡	0.43	5.76
anteiso 15:1	0.04	0.48	18:4‡	0.76	10.1
16:1‡	29.2	390	20:4‡	8.39	112
16:1‡	0.55	7.36	22:4‡	0.25	3.37
iso 16:1†	0.06	0.83	20:5‡	7.30	97.8
17:1‡	2.14	28.7	22:5‡	0.12	1.66
17:1‡	0.45	6.02			
iso 17:1†	0.25	3.29	t16:1‡	0.27	3.56
anteiso 17:1	0.09	1.17	t16:1‡	0.47	6.32
18:1‡	1.88	25.2	t18:1‡	0.14	1.89
18:1‡	3.30	44.2	t18:1‡	0.11	1.51
16:2‡	1.26	16.9			
16:2‡	0.87	11.6			

* Data quoted to two decimals is only used to indicate the ratio of minor components. Actual accuracy is about 15% and the values are corrected for the slight loss of unsaturated acids on the capillary columns. † Two unidentified isomers. ‡ Mainly Δ^9 . § Trace $\omega 3$ and $\omega 6$ isomers.

in each case were *trans*. In our sediment the percentage of the *cis* isomer is larger (99% for 16:1, 95% for 18:1) and the proportion of *cis* and *trans* for each positional 16:1 and 18:1 isomer varies significantly. The range of *trans* acids in the sediment seems to parallel the range of the corresponding *cis* isomers but the very low concentrations encountered here have prevented us from obtaining unequivocal identifications. These acids and the change of the *cis-trans* ratio with depth are under study. The other *trans* isomer isolated, t16:1‡, is probably of biological origin. This acid has not previously been reported from a sediment, despite its ubiquity in photosynthetic organisms where it is found localised in the chloroplast phosphatidyl glycerol fraction¹⁴. This acid may prove to be a good marker for a contribution from photosynthetic organisms.

This report shows that a detailed study of the gross fatty acid patterns can be combined with the isomeric forms of these acids to be of value to the environmentalist in relating isolates to probable origin. In our case diatoms and marine bacteria are strongly implicated as the major contributors of organic carbon to this typical, temperate intertidal zone sediment.

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Are monkeys logical?

THE monkey's status as a thinker has never been high; yet laboratory investigations testify, nevertheless, to the ability of many species of monkey to learn complex tasks, if not to reason. On this latter point, however, hard evidence is significantly lacking. One reason for this is that it is difficult to devise tests which are both meaningful to non-verbal subjects yet satisfy the stringent requirements of a formal reasoning test such as one adapted from Burt¹ which first gives the subject the following information: "Edith is fairer than Suzanne", "Edith is darker than Lili", and then requires solution of the question, "which is the darkest, Edith, Suzanne or Lili?". Bryant and Trabasso² have devised a simplified method of giving such tests to very young children, and we have adapted this into a non-verbal one for use with monkeys.

In Bryant and Trabasso's study, children were first trained to label five rods presented in four subsets as big or small according to the colour and the subsets in which they were embedded. As displayed, however, no size differences were apparent. Thus, for example, a yellow rod (A) was taught as 'taller than' a red one (B), a red one as 'taller than' a blue one (C), a blue rod was learnt as 'taller than' a green one (D), and a green one as 'taller than' a white one (E). On subsequent tests, children were asked to judge the relative sizes of non-adjacent members of the set, for example red (B) against green (D).

Eight adult squirrel monkeys were tested in an apparatus (a Visconsin General Testing Apparatus) which allowed the monkey to view on any one trial, a tray bearing two cylindrical tin containers of equal size but differing in colour and weight. The experimental design is depicted in Table 1. Each monkey was confronted with four pairs of colour discriminations, learnt serially. For half the subjects the rewarded stimulus was heavier than the non-rewarded one; for the other half it was the lighter one. Weight differences were used to emphasise stimulus differences in the course of preliminary training³. Only two weight values were used throughout the experiment, however; the 'heavy' tin in any pair was filled with lead shot, the 'light' one was empty. Thus, no specific weight could be uniquely identified with stimuli B, C and D.

Monkeys were assigned to different colour pairings and the tray colour was also varied from monkey to monkey in an attempt to eliminate fortuitous colour preferences which might affect the crucial test choices. Each pair was

Table 1 Scheme of training paradigm

Series identification		A	B	B	C	C	D	D	E
Pair		1		2		3		4	
Weight	4 subjects	Light	Heavy	Light	Heavy	Light	Heavy	Light	Heavy
Colour (for example)	4 subjects	Yellow	Blue	Blue	Green	Green	Red	Red	White

learnt in serial order from AB to DE to a criterion of 18/20 trials correct for each pair. Choice of one of the tins was rewarded with a peanut located in one of two foodwells directly underneath the stimuli. To indicate choice and secure the reward, the monkey had to manually displace the appropriate tin from its position over the foodwell. After an error, however, the monkey received no reward; the tray was withdrawn in full view of the subject for 5 s, and a screen was then lowered to permit the experimenter to replace the stimulus unseen and restart testing. The respective positions of the stimuli varied from the left to the right of the tray according to a random sequence.

When subjects had learnt all four pairs they were given a second run through all problems in the same order to the same performance criterion. Several subsequent runs then followed in which the number of training trials given per problem was progressively reduced. Finally, five runs were given of one trial per problem in a random order of presentation. A maximum of 50 trials per day was administered. When each monkey had progressed through all stages of the procedure outlined above, it was given a series of 10 critical trials on the B against D comparison (see Table 1). 'Critical' tests, however, were not administered in any session until the animal first recorded a performance of 22/24 correct in the course of 24 successive encounters of training pairs in a random sequence; not more than two transitive tests were administered per session. All choices were rewarded irrespective of bias during the testing phase. When the B against D tests were complete, subjects were given further tests in similar conditions with the AC, AD, AE, CE and BE combinations in counterbalanced order of presentation (these are regarded as less critical as they include stimuli A and E which can be identified as having been invariably rewarded (E) or invariably non-rewarded (A)). All but one monkey learnt the series; the eighth was erratic and did not meet our stringent training criteria sufficiently often to warrant subsequent testing.

In Table 2 the monkey results are compared with the child data obtained by Bryant and Trabasso for 4-yr-old children. These data clearly show a choice profile consistent with the notion that monkey choices are transitive and accord in virtually every detail with the data reported for children.

Are we to assume, therefore, that monkeys solve such problems by means of deductive inference? Certainly the evidence is consistent with the idea that in order to solve such problems, subjects must coordinate two vital pieces of information—for example that C is heavier than B, and that D is heavier than C. There are alternative ways in

which the problem might be solved, however. Among these⁴, the one which is perhaps the most radically at odds with the coordination model¹ assumes that transitive choices result from single binary decision making.

A set BCD, for example, affords three subsets, BC, CD and BD. If a decision is based exclusively on the interrogation of any one subset, and if we assume (in the absence of information to the contrary) that each subset is interrogated equally often, then it is possible to compute the choice proportions to each of the constituent members of the triad BCD on a given trial. If, therefore, the probability of selecting any one of the subsets as a basis for choice is 0.33 (approximately) we can thus predict an overall choice proportion of 0.33 in the case of C (assuming absolute preference for C over B). D has two reference subsets, CD and BD. In the case of subset CD (making the same assumptions as above) the choice proportion predicted for D will be approximately 33%. In the case of subset BD, however, we need assume no consistent preference for either B or D. On the 33% of all occasions on which these comparisons are made, the subject may select either one equally often. In this case, the overall probability of choosing D would rise by a further 16% (with roughly 16% of all choices going to B). Thus we might predict the approximate choice proportions for all three stimuli in the set BCD as follows: B = 0.17, C = 0.33 and D = 0.50.

In a two-choice situation, of course, where B is presented in conjunction with D, C would have to be 'inferred' as a referent (an assumption in common with the coordination model, some theories of perception⁵, and of transitivity⁶ and a reasonable one given the duplicate relationship of C with B and D). In this instance the choice proportions attributable to C when actually present will now add to the overall proportions for D (as half of them will rule out responses to B, the other half will confirm D directly). Thus the probability values for BD in a two-choice situation will now be B = 0.17, D = 0.83.

In the case of comparisons involving an endpoint value, however, (either A or E), fewer subsets will be at chance levels of choice (taking as a total population all 10 triadic permutations of the 5 term series A, B, C, D and E); thus the projection for the average transitive choice proportion is 0.98. Such predictions seem a remarkably close fit to data already obtained in various experiments reported to date (Table 3).

Apart from fitting predictions to existing data, however, to provide an alternative account to the coordination model, there is an empirical test of the relative strengths of these opposed accounts which is made possible if three

Table 2 Transitive choice data for squirrel monkeys compared with the profile reported by Bryant and Trabasso⁴ for 4-yr-old children.

Monkeys					4-yr-old children (B + T) (Experiment 1)				
	B	C	D	E		B	C	D	E
A	0.98	1.00	1.00	1.00	A	0.96	0.96	0.93	0.98
B	—	0.93	0.90	0.76	B	—	0.92	0.78	0.92
C	—	—	0.89	0.87	C	—	—	0.90	0.94
D	—	—	—	0.97	D	—	—	—	0.91

All choices reported for monkeys are significant on a binomial test ($P < 0.001$). Note especially the B against D comparisons which cannot depend directly on 'end-anchoring' effects.

Table 3 Averaged transitive choice data taken from three separate investigations (choice proportions)

Investigation	Choice proportions to pairs with end points	Proportions to the 'critical' pair (BD)	Overall transitive choice proportion
Bryant and Trabasso ² experiments 1 and 2 (5 groups)	0.96 (0.98)	0.85 (0.83)	0.94 (0.96)
de Boysson-Bardies and O'Regan ⁷ experiments 1 and 2 (2 groups)	0.91 (0.98)	0.82 (0.83)	0.90 (0.96)
This work (1 group)	0.93 (0.98)	0.90 (0.83)	0.92 (0.96)
Average (all investigations)	0.95 (0.98)	0.85 (0.83)	0.93 (0.96)

Values in parentheses are predicted values from a binary choice model (basis of computation outlined in Table 4).

(or more) stimuli, for example B+C+D, are actually presented to the subject for choice following establishment of strong preferences within subsets BC and CD. On the coordination model it is not readily apparent why the actual presentation of the three relevant terms would do other than emphasise further the choice bias in favour of D obtained in the course of two-choice transitivity tests (for example BD). On the binary sampling model, however, a 'reduced' transitive effect is demanded whereas no such predictions are afforded by the coordination theory. We report below a direct test of these positions.

The seven remaining monkeys were presented with 10 triplets derived from all possible triadic combinations of the five stimuli in the original set (A, B, C, D and E). Each session began with 25 trials involving the original training pairs presented in random order. If not more than two errors were recorded, monkeys were then confronted with a three-choice situation where, for another 25 trials per session, each of the 10 triplets was presented in a counterbalanced order. The position of each stimulus on the tray was also counterbalanced. The stimuli were either all 'heavy' or 'light' in accordance with the previous training history of the animal. All choices were rewarded, and the experiment was terminated when subjects made 10 separate choices to each of the 10 triplets presented for test.

Table 4 Profile of choice following triadic presentations

Triads	Choice projection*			Obtained		
ABC	00	33	67	00	31	69
BCD	17	33	50	03	36	61
BDE	17	17	67	16	24	60
CDE	00	33	67	11	24	65
BCE	00	33	67	06	28	66
ABD	00	50	50	00	44	56
ACD	00	33	67	00	30	70
ADE	00	33	67	01	21	78
ABE	00	33	67	00	30	70
ACE	00	33	67	00	26	74
Average distribution	0.03	0.33	0.64	0.04	0.29	0.67

The figures in the left-hand column are predictions based on the assumptions that the subsets are sampled equally often and that preferences are absolute within those subsets presented during original training.

*Basis for predictions in Table 3.

Table 4 shows the overall choice profile in these conditions, and shows how the response distributions for triads are well predicted from a binary (statistical) decision model. By contrast, a coordination model² does not readily lend itself to any quantifiable prediction of a reduced transitive effect. On the contrary, with the relevant choice items in the immediate perceptual field, the 'transitive' choices should, if anything, be all the more pronounced.

Whatever the case for, or against, deductive reasoning which may be fashioned from results such as those reported here, it is clear that some kind of 'inference' necessary to produce the appropriate 'inferred' set or absent referent is used by monkey in tests of transitivity such as those described here. Whether such operations (of 'inference')

provide for the foundations of logical development remains unknown.

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Dissolved ATP in the sea and its utilisation by marine bacteria

THE biologically labile fraction of the dissolved organic matter (DOM) in the oceans seems to be a chemically complex solution of a wide variety of compounds derived mainly from the contents of cellular metabolic pools released into the seawater after the death and cell lysis of marine organisms. This fraction is of particular geochemical interest because of its importance in understanding the cycling of organic matter in the marine environment. Because of methodological problems arising from the extremely low concentrations of individual compounds, compound-specific analyses have been limited to only a few components of the DOM, such as amino acids¹, lipids², sugars³, and vitamins⁴. Adenosine-5'-triphosphate (ATP) would be a useful tracer for following the production and fate of labile DOM in seawater as it is a universal component of the cellular metabolic pools of all living organisms. We report here that dissolved ATP (DATP) occurs in seawater in significant concentrations (0.1-0.6 $\mu\text{g l}^{-1}$) and is utilised rapidly by marine bacteria.

Although the particulate ATP content of marine seston (suspended particles) has been used extensively as a sensitive indicator of microbial biomass in oceans and lakes⁵, the presence of DATP in seawater had not been demonstrated conclusively⁶. This has been due, in part, to the absence of a suitably sensitive method, and, in part, to the general assumption that ATP is so rapidly degraded by transphosphorylases and ATPases after cell death as to preclude its presence in the DOM. We considered that the rate of enzymatic breakdown of DATP in the sea might be negligible because extreme dilution of both the enzymes and the substrate occurs after cell disruption.

DATP concentrations in coastal waters of Southern California and British Columbia, Canada were measured using a modification of the method of Hodson *et al.*⁶, which was developed originally for determination of ATP associated with organisms in marine sediments. Seawater samples (50 or 100 ml) were first filtered through 0.2 μm Nuclepore filters to remove ATP associated with organisms and other particles. Filtrates were acidified to 0.6 N with sulphuric acid. A trace amount of $\gamma\text{-}^{32}\text{P}\text{-ATP}$ (less than 1 nCi per sample; 700 Ci mmol⁻¹) was added as an internal standard and the sample was passed through a column of activated charcoal. Adsorbed ATP was then eluted with

5 ml ammoniacal ethanol (ethanol—ammonia—water, 2:2:1 v/v), dried *in vacuo* at 40 °C, and dissolved in 1 ml Tris (tris hydroxymethyl aminomethane) buffer (pH 7.7, 20 mM). Percentage recovery of ATP was determined by radioassaying the concentrates without adding fluor, by Cerenkov Radiation, so that the same sample could be used to determine ATP. ATP was measured by the firefly luminescence method⁴.

It was possible that DATP in seawater samples was an experimental artefact, and that it was introduced into filtrates by cell lysis during vacuum filtration. This was found not to be the case by comparing the filterable ATP with dialysable ATP. Cleaned dialysis bags (molecular weight cut-off 12,000–14,000) were filled with double-distilled water and autoclaved. These dialysis bags were suspended from the Scripps Institution of Oceanography (SIO) pier to a depth of 1 m for 4–24 h. The contents were removed with a sterile syringe and hypodermic needle, and assayed for ATP as above. DATP concentration in the dialysis bags was not appreciably different from that in filtrates ($\Delta P = 5$ cmHg). Another check was made by filtering aliquots of seawater samples using a range of pressure differentials (2.5–37.5 cmHg) and measuring DATP in the filtrates. No increase in DATP resulted with increasing ΔP .

The concentration of DATP in the relatively oligotrophic surface seawaters off the coast of Southern California varied from an average high value of 218 ng l⁻¹ off the SIO pier to an average low value of 65 ng l⁻¹ at a distance of 4 miles seaward from Point Loma, San Diego, California. In the highly eutrophic waters of Saanich Inlet, British Columbia, the DATP concentration averaged 466 ng l⁻¹. The distribution of DATP with depth was measured at a station 16 miles offshore from San Diego in the San Diego Trough (Fig. 1). The DATP concentration in the euphotic zone averaged 110 ng l⁻¹, decreasing to a uniform value of 40 ng l⁻¹ in the aphotic zone down to a depth of 500 m. At this depth, the ATP content of the particles, including the living organisms, was only 4 ng l⁻¹, which is typical of these waters⁴.

Possible sources of the DATP in surface seawater include phytoplankton mortality due to zooplankton grazing and the attendant release of soluble metabolic pools, and the excretion of ATP by plankton. Possible sources of DATP in the deeper parts of the water column are oceanic mixing processes, and the sinking of faecal pellets, clay minerals, and other particles which may contain associated ATP.

Three possible mechanisms of DATP turnover were examined: spontaneous ATP hydrolysis in seawater, enzymatic hydrolysis by extracellular ATPases, and biological uptake and assimilation. ATP was stable in seawater that had been filtered through 0.2 μ m Nuclepore filters to remove all organisms and then autoclaved (15 min at 121 °C) to denature any ATPases; no loss was detectable after 24 h at 21 °C. The stability was not determined over longer periods of time, but previous work has shown that the half life of ATP in artificial seawater at 21 °C is about 8 yr (ref. 7).

Enzymatic hydrolysis of DATP in seawater was examined by filtering seawater samples through 0.2 μ m Nuclepore filters and following the rate of ATP loss at 21 °C; no detectable loss was observed after a 24 h period.

Rapid turnover of DATP occurs when living organisms are not removed from seawater samples. Turnover rates were determined by measuring the assimilation of 2,8-³H-ATP by the natural microbial populations present in the samples. Controls, which received 1% formalin before addition of ³H-ATP, accumulated (adsorbed) ³H at less than 5% of the experimental samples. Using U-¹⁴C-ATP as tracer, it was determined that more than 98% of the ¹⁴C taken up was assimilated, whereas only an insignificant fraction was respired. Turnover rates varied as expected, but were quite rapid in all samples, with rates

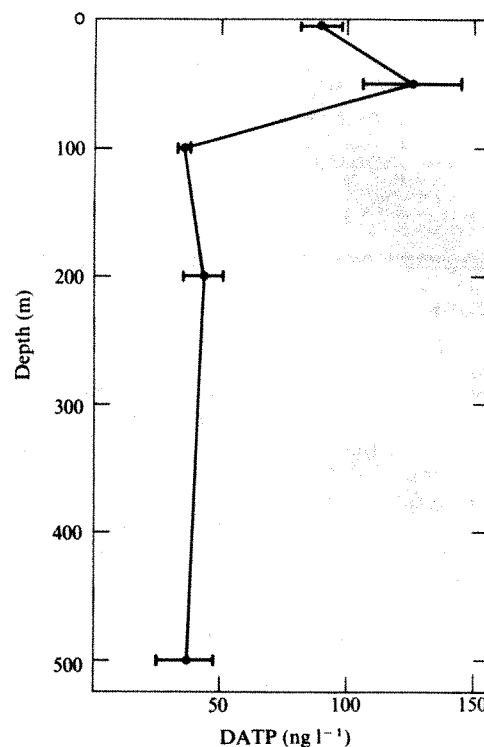


Fig. 1 Dissolved ATP concentration as a function of depth from 32°41.6'N; 117°22.7'W in San Diego Trough.

as high as 15.2% h⁻¹ in a sample collected in Saanich Inlet, British Columbia; the range was 0.59–15.2% h⁻¹.

To estimate what part of the ATP uptake was due to bacteria, the seawater samples were first incubated with ³H-ATP and then equal aliquots were filtered on 0.2 μ m and 0.6 μ m Nuclepore filters, and the ATP assimilation by total and 0.6 μ m filterable organisms determined. It has been shown previously that the assimilation of radiolabelled organic compounds from seawater by 0.6 μ m filterable particles can be treated as being of bacterial origin⁴. About 80% of the assimilation of DATP was bacterial; the rest is assumed to be due either to algae and micro-zooplankton; or to bacteria attached to larger particles. We have previously shown that bacteria are responsible for 80–90% of the assimilation of dissolved D-glucose, L-serine, and acetate from seawater⁵.

Twelve isolates of marine bacteria were examined for their ability to assimilate 2,8-³H-ATP. Eleven of these isolates were highly efficient in assimilating DATP. Bacterial growth in the sea is characterised by extreme limitation of carbon and energy sources, individual growth substrates being present at nanomolar concentrations. The presence of a significant concentration of DATP which is rapidly turned over suggests that ATP may be an important carbon and energy source for marine bacteria. Its ubiquitous presence in seawater and the fact that its concentration can be determined with great sensitivity (10⁻¹¹ M), may make the measurement of its flux into marine bacteria a useful indicator of bacterial heterotrophic activity in a given body of seawater.

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Sex pheromone of the California red scale, *Aonidiella aurantii*

THE California red scale, *Aonidiella aurantii* (Maskell), is the most important citrus pest in Australia, California and the Mediterranean countries. It has been found¹ that red scale females attract males with a sex pheromone, and preliminary studies² have indicated that a branched acetate may be involved. Much interest has been generated in the identification of this pheromone because no other Homopteran sex pheromone has been reported and because the synthetic pheromone has immediate application in replacing the commercial virgin female traps used extensively in monitoring this pest. The sex pheromone reported here consists of two novel components, 3-methyl-6-isopropenyl-9-decen-1-yl acetate (I) and (Z)-3-methyl-6-isopropenyl-3, 9-decadien-1-yl acetate (II). The norsesquiterpene structures represent interesting biosynthetic pathways since they do not merely involve head-to-tail coupling of the isoprenoid units. These compounds also present a synthetic challenge because (I) possesses two asymmetric centers and (II) possesses one asymmetric centre (Fig. 1).

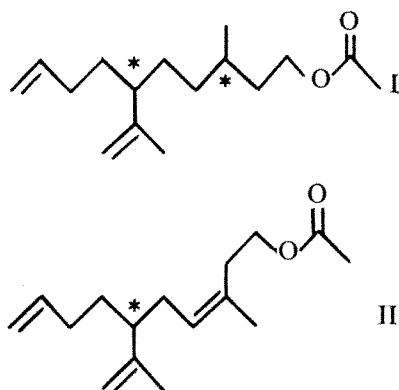


Fig. 1 Components of sex pheromone from California red scale.

Crude pheromone was first obtained from condensates from air that had passed over scale-infested lemons into a dry-ice trap. Steam distillate from the aqueous condensate was extracted with ether-hexane (3:1). An improved system later utilised a Porapak Q trap³ and scale-infested potatoes. A total of approximately 400 million female-day-equivalents (FDE) were collected. An FDE is the amount of pheromone collected from one female each day.

Pentane extracts of the Porapak were concentrated and the residue was chromatographed on a Florisil column (1.5 × 50 cm) eluted with 900 ml of a 0-50% gradient of ethyl ether in Skelly B. The fractions (15 ml) were tested for activity in a greenhouse bioassay⁴, in which test materials on filter paper disks were positioned on a 1.8-m diameter turntable, and red scale males were selectively captured on sticky cards (7.5 × 12.7 cm) placed above each sample. A typical test with Florisil fractions resulted in captures of several hundred males with fractions 14-20 and less than three males with any other fraction.

Active fractions were combined and chromatographed on a 10% AgNO₃-Bio-Sil A (20-44 μm) HPLC column (1 × 100 cm)

eluted with 500 ml each of 10% ether and 20% ether in benzene. Bioassay results showed activity in fractions (15 ml each) 9-14 and 29-38. Each set of active fractions was combined and labelled AI and AII, respectively.

AI and AII were purified further by elution from a Lichrosorb HPLC column (0.2 × 50 cm) with 20 ml 1% ethyl acetate in heptane (0.5 min fractions). All activity was collected during the period 7.5-8.0 min for both samples. Collections from gas-liquid chromatography (GLC) columns showed that the activity was associated with one visible peak in each case, with carbon numbers relative to saturated acetates as follows: 3% OV-101 (AI = 12.25; AII = 12.06); 10% XF-1150 (AI = 13.08; AII = 13.00). The components were purified finally by collection from OV-101 to provide approximately 50 μg of each.

The presence of an acetate moiety was indicated by reaction of AII with LAH to yield an inactive product. Activity was restored at the original GLC retention time on OV-101 by treating the product with acetyl chloride. Mass spectra of AI and AII showed M-60 fragments at 192 and 190 m/e, respectively, possibly corresponding to doubly and triply unsaturated 14-carbon acetates. Hydrogenation produced the same product with an M-60 fragment at 196 m/e from both AI and AII; this was converted to the corresponding hydrocarbon skeleton by saponification, substitution of the hydroxyl group with bromine and reaction with LAH. This hydrocarbon exhibited a molecular ion at 198, a major fragment at 155 (M-43), and typical hydrocarbon fragmentation to the base peak at 57 m/e. Ozonolysis of AI did not yield any GLC visible product, but ozonolysis of AII produced an isolable compound identified as 3-ketobutan-1-yl acetate by GLC and mass spectral comparisons with a standard.

The PMR spectrum of AI (C₆D₆; 100 MHz) was consistent with structure I and has δ values as follows: 5.75 (m), 5.07 (d), 5.02 (d) [H₂C=CH-CH₂-]; 4.82 (s) and 4.76 (s) [R₂C=CH₂]; 4.09 (t) and 1.7 (s) [-CH₂-O-CO-CH₃]; 1.95 (m) [3 allylic H]; 1.49 (s) [CH₃C(R)=]; 1.1-1.6 (m) [9 saturated H]; and 0.77 (d) [-CH-CH₃].

The spectrum of AII (CS₂; 300 MHz) was consistent with structure II and has δ values as follows: 5.71 (m), 4.92 (d), 4.87 (d) [H₂C=CH-CH₂-]; 5.12 (t) [R₂C=CH-CH₂-]; 4.7 (s) and 4.64 (s) [R₂C=CH₂]; 3.95 (t) and 1.92 (s) [-CH₂-CH₂-O-CO-CH₃]; 2.27 (t) [C=C(R)-CH₂-CH₂-]; 1.9-2.1 (m) [5 allylic H]; 1.69 (s) and 1.60 (s) [2 CH₃ C(R)=C]; and 1.39 (m) [2 saturated H].

A 1:1 mixture of E and Z isomers of compound II was prepared from either (S)-(+)-carvone or (R)-(-)-carvone. The former produced the R enantiomer (R:S ratio was 95.5:4.5) and the latter produced the S enantiomer (S:R ratio was 96:4). The geometrical isomers were easily separable on several GLC columns, and were characterised by the chemical shifts (CS₂; 100 MHz) of the 3-methyl proton singlet at 1.69 and 1.60 p.p.m. for the Z and E isomers, respectively. The natural compound AII had GLC retention times identical to the synthetic Z isomer on OV-101 and XF-1150, with no evidence for the E isomer. The proton magnetic resonance spectrum of synthetic II (CS₂; 100 MHz) was similar to that of the natural compound (CS₂; 300 MHz), and mass spectra were identical for AII and II, for their hydrogenated products, and for the hydrocarbon skeletons obtained from each.

Greenhouse flight tests showed that the (R)-Z isomer (25-130 ng on filter paper) is competitive with 25 virgin females, whereas the other isomers are inactive. A typical test (25-ng sample, two replicates for two nights) gave male catches of 531 for the (R)-Z isomer and 15, 17, 18 and 15 for the (R)-E, (S)-Z, (S)-E isomers, and the blank, respectively. Preliminary field tests confirmed the strong attractancy of synthetic II (R-Z) compared with standard virgin female traps (200 females per trap). Field studies will be carried out on all enantiomers of II, as well as with those of I when available.

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Thermal selection of allozyme polymorphisms in barnacles

Is protein polymorphism adaptive or neutral? Attempts to assess the neutrality theory on the basis of gene frequencies and theoretical population genetics models seem, at least to some authors, to be unsuccessful¹. Possible promising approaches involve the search for direct correlation of isozymes with the environment² and with physiological function³. We have tested the effects of temperature on barnacles, because temperature affects protein structure and function^{4,5,6}, as well as size⁷, and because adult barnacles, following larval settlement, are stationary and directly exposed to local temperature variation. Our results suggest strong temperature selection on allozymic and size variations in the acorn barnacle, *Balanus amphitrite*.

Acorn barnacles, genus *Balanus*, are marine crustaceans, with almost worldwide distribution. Our sampling areas were the open Mediterranean seawater canals of the Haifa Electrical Plant Cooling System, where temperature seems to be the only environmental factor distinguishing the cooler inflowing and warmer outflowing canals (about 12 °C and 9 °C mean difference in summers 1975 and 1976, respectively; see Tables). We compared *B. amphitrite* from the cooler and warmer canals for numbers, size, and for allozyme variation at

11 scorable gene loci in 1975 and at 8 differentiating loci in 1976. To compare equal age populations in the cooler and warmer canals, we immersed 625 cm² plastic plates, 100 cm below water surface, in May and removed them in October. A sample of 260 barnacles—six samples, three from the inflowing and three from the outflowing canals—were taken in three repetitive tests, one in 1975 and two in 1976 (from two different pairs of canals, see Tables). Animals that settled on the plates in each outflow have all passed through the relevant inflow. Soft tissues of whole animals were extracted from the calcareous shells, homogenised, and studied using starch gel electrophoresis⁸ for allozymic variation encoded by the following 11 gene loci: phosphoglucose isomerase (*Pgi*); malic enzyme (*Me*); acetaldehyde oxidase (*Ao*); two loci; *Ao-1* and *Ao-3*; malate dehydrogenase (*Mdh-1*); acid phosphatase (*Acph-1*) and esterases (five loci: *Est-1*, *Est-3*, *Est-4*, *Est-7* and *Est-8*).

The number of barnacles per plate (on both sides of the plate) in the inflowing canals was about 2,400 compared with 510 per plate in the outflowing canals. The average size of an individual in a sample from the inflowing canals was 336 mm² (s.d. 104), compared with 135 mm² (s.d. 47) in the outflowing canal ($n_1 = n_2 = 30$; $t = 9.65$; $P < 0.001$). Out of 11 isozymes tested in 1975, three (*Ao-1*, *Est-1* and *Est-7*) showed no differences between the cooler and warmer canals, and were therefore discarded. The remaining eight showed distinct repetitive trends in the 1975 and 1976 tests. First, the estimates (Table 1) of heterozygosity (h) for individual loci decreased significantly in four loci (*Me*, *Acph-1*, *Est-3* and *Est-4*), most remarkably in *Me*. Mean heterozygosity for all 8 loci (H) in the three tests decreased significantly from 0.110 in the cooler to 0.047 in the warmer canal ($P < 0.001$). Second, distinct statistically significant repetitive trends (Table 2), in allele frequencies were found for chosen alleles in seven out of the eight loci. The following alleles increased in frequency in the warm canal: *Me*^{M+}, *Ao-3*^F, *Mdh-1*^F, *Acph-1*^M, *Est-3*^F, *Est-4*^F and *Est-8*^F; there was a non-significant increase in *Pg*^S. Significant deviations from Hardy-Weinberg expectations due to heterozygote deficiencies were found for *Me* in both the cooler and warmer canals ($\chi^2_{(1)} = 10.04$, $P < 0.01$; $\chi^2_{(1)} = 79.6$, $P < 0.001$, respectively), and for *Est-3* in the warmer canal only ($\chi^2_{(1)} = 97.8$, $P < 0.001$).

To obtain an approximate measure of the favoured allele we assume fitnesses W_1 , W_2 , W_3 for *AA*, *Aa* and *aa*, respectively, where a denotes the favoured allele in the warmer canal, and A all the remaining alleles. The relative fitnesses of *aa* compared with *AA* (W_3/W_1) in the warmer compared with the cooler canal for *Me*, *Acph-1*, *Est-3* and *Est-8*, were 3.6, 2.2, 21.3, and 4.4, respectively. Evidently, strong directional selection is

Table 1 Estimates of heterozygosity (h) for each of eight loci in *B. amphitrite* from the inflowing and outflowing canals at the Haifa Electrical Plant Cooling System

Month and year:	October 1975					October 1976					October 1976					Pooled Z
No. of test	1					2					3					
Canals	In		Out		Z ⁴	In		Out		Z	In		Out		Z	
Temperature (°C, mean) ¹	24.1	36.6	24.6	33.1		(Roughly as in test 2)	24.1	36.6	24.6		33.1	24.1	36.6	24.6		33.1
Locus	n ²	h ³	n	h		n	h	n	h		n	h	n	h		
<i>Pgi</i>	29	0.103	28	0.143	—	71	0.042	68	0.103	—	29	0.034	30	0.167	—	
<i>Me</i>	28	0.464	29	0.172	2.37**	72	0.774	65	0.187	6.86†	29	0.724	30	0.133	4.59†	
<i>Ao-3</i>	29	0.000	30	0.000	—	72	0.000	68	0.029	—	28	0.036	30	0.000	—	
<i>Mdh-2</i>	21	0.048	18	0.056	—	72	0.042	68	0.059	—	29	0.000	30	0.067	—	
<i>Acph-1</i>	29	0.000	29	0.000	0.00	72	0.056	64	0.000	1.92*	29	0.103	26	0.038	0.93	
<i>Est-3</i>	27	0.148	26	0.038	1.37	71	0.085	59	0.000	2.29*	28	0.000	27	0.000	0.00	
<i>Est-4</i>	29	0.172	29	0.034	1.73*	71	0.028	66	0.000	1.37	29	0.103	29	0.034	1.04	
<i>Est-8</i>	29	0.000	29	0.069	—	72	0.000	64	0.016	—	29	0.000	28	0.000	—	
Mean	H ⁵	0.095	*	0.026		0.114	*	0.055			0.111		0.041		**	

¹ Mean temperature calculated from daily minimum–maximum recordings.

² n, Number of barnacles tested.

³ h, Heterozygosity at each locus.

⁴ Z, Statistic comparing proportions with a specified characteristic with two independent samples ($Z = \sqrt{\chi^2_{(1)}}$).

Wherever Z values do not appear in body of Table, h in the outflowing canal is higher than h in the inflowing canal. Since the test is one-sided these results are obviously non-significant.

⁵ H in the cooler and warmer canals were compared by the Mann–Whitney test, carried out on the observed numbers of heterozygotes over the eight loci in each individual. Thus, it is impossible to perform it directly on the data as they appear in this Table. The P values were <0.05 for test 1, <0.01 for test 2, <0.001 for test 3.

Levels of significance: *P < 0.05; **P < 0.01; †P < 0.001.

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Electrical responses of *Stomatoca atra* induced by an extract of *Aequorea aequorea*

HYDROMEDUSAE swim by rhythmically contracting the circular swimming muscles located in the subumbrella. When species such as *Stomatoca atra*¹, *Sarsia tubulosa*^{2,3}, *Proboscoidactyla flavicirrata*⁴ and *Euphysa japonica*² are disturbed, either by electrical or mechanical stimulation of the exumbrellar surface, the tentacles contract, swimming stops and a tetanic contraction of the radial muscles causes an infolding of the bell margin (Fig. 1b). This protective response is called crumpling⁵ and is initiated by all-or-nothing potentials in the nerve-free, exumbrellar epithelium^{1–4}. *S. atra*, a predacious hydromedusa, is atypical in that it crumples both when disturbed and when in the proximity of large numbers of hydromedusae of several other species. The latter response is initiated by a stimulating substance stored in the mesogloea of the other medusae⁶. The substance is found in crude extracts of *Aequorea aequorea*, *Halistaura celluaria*, *Phialidium gregarium* and *S. tubulosa*, but not in *S. atra* itself. The crumpling response induced by the extract has been suggested to be a feeding response, trapping prey in the infolded margin⁶. By recording the electrical activity during the crumpling response initiated by extracts of *A. aequorea* I have found that this response is coordinated differently from crumpling initiated by electrical or mechanical stimulation.

Suction electrodes placed on the subumbrellar surface of the animal monitored electrical events associated with swimming or crumpling. A crude extract of the stimulating substance contained in *A. aequorea* was prepared by mincing an intact animal with a scalpel blade and collecting the resulting fluid. To induce crumpling a few drops of the extract were added to a chamber containing a specimen of *S. atra* swimming in 4 ml of seawater at 11 °C, the local

Fig. 1 Silhouettes of *S. atra* drawn from photographs. a, At rest. b, During a crumpling response induced by mechanical agitation (MIC). c, During a crumpling response induced by the extract of *A. aequorea* (SIC).

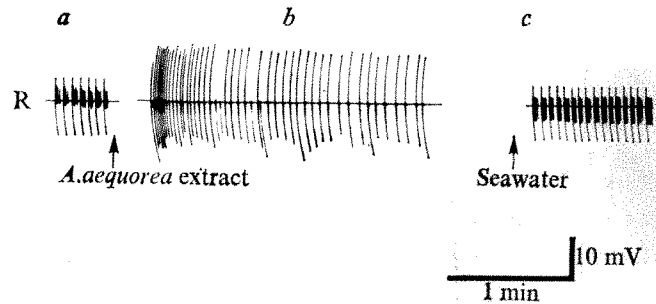
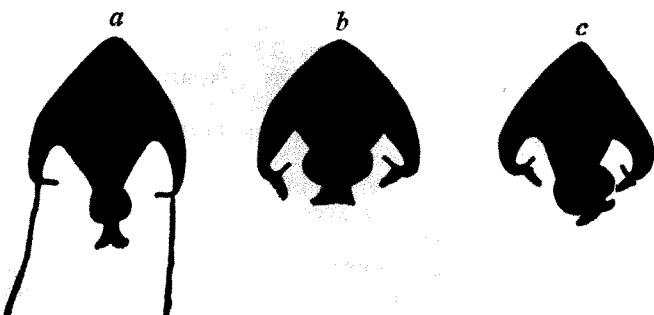


Fig. 2 Electrical activity recorded from the subumbrella of *S. atra*. a, Potentials recorded during swimming. b, Crumpling potentials induced by the *A. aequorea* extract. c, Recovery of swimming potentials after the medium was replaced by fresh seawater.

temperature of seawater. Drops of seawater added to the specimen instead of the extract served as a control for all experiments and in no case did seawater induce crumpling. Normally *S. atra* swims in bursts, but in the presence of the extract, the tentacles contracted, swimming and the associated electrical activity ceased and crumpling pulses⁴ were recorded from the subumbrella (Fig. 2). If the solution containing the extract was replaced by fresh seawater the crumpling pulses stopped, the radial muscles relaxed and swimming resumed (Fig. 2).

Since normal crumpling is initiated by propagated potentials in the exumbrellar epithelium, it was important to determine if the extract initiated such electrical events. *A. aequorea* extract did not excite the exumbrellar epithelium of either intact animals or excised pieces of bell containing only exumbrellar tissue. Excised pieces of bell were used as a precaution against recording potentials from tissues deeper than the epithelium directly beneath the active electrode³. The exumbrellar epithelium in both intact animals and excised pieces of tissue did conduct propagated potentials when stimulated electrically and conduction velocity between two electrodes was unaffected by the extract.

The extract not only causes a cessation of swimming and an infolding of the margin but also manubrial flexion⁶, a behaviour not observed in the electrically or mechanically induced crumpling response¹ (Fig. 1b, c). Manubrial flexion is called pointing and is caused by the contraction of one or two of the four radial muscles⁷. Isolated manubria, which included a portion of the peduncle, pointed in the presence of the extract. Suction electrodes placed over a radial muscle, in such a preparation, showed that each flexion was associated with a burst of potentials different from crumpling pulses (Fig. 3). Manubrial flexion stopped when the extract was flushed out with fresh seawater.

The extract-initiated behaviour is similar to normal crumpling but there are differences between the two responses which indicate that they are coordinated differently. I shall refer to the electrically or mechanically induced crumpling as MIC and crumpling initiated by the stimulating substance contained in some hydromedusae as SIC. *S. atra* has six described conducting pathways and MIC has been shown to involve the swimming and tentacle pathways, which are neuronal, and the exumbrellar and lamellar pathways, which are non-neuronal¹. The crumpling pulses initiated by the extract and recorded from the subumbrellar surface were similar in amplitude and duration to the pulses recorded during MIC from the same tissue by Mackie¹. Crumpling pulses recorded from the subumbrella during MIC originate in the endodermal lamella^{1,2}, and by analogy, the crumpling pulses recorded from the subumbrella during SIC are also conducted in the non-neuronal lamellar pathway. Further, the similarity in tentacle shortening in MIC and SIC indicates that the

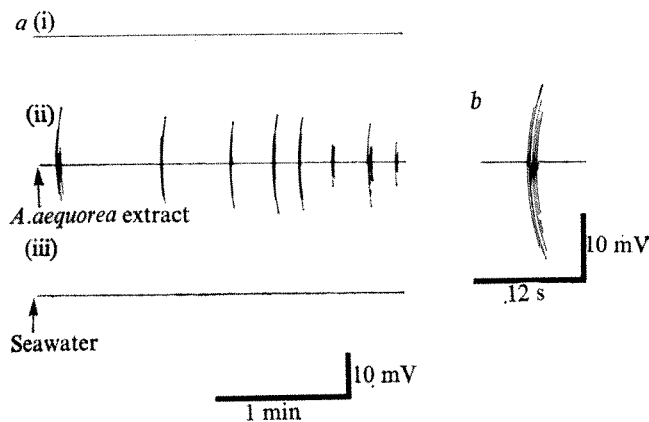


Fig. 3 *a*, Electrical activity from the area of a radial canal in an isolated manubrium (i) before, (ii) during and (iii) after the application of the extracts. *b*, Shows the multiple events associated with the pointing response induced by the extract.

tentacle pathways are used in both behaviours. But MIC and SIIC differ in two aspects. First, SIIC does not utilise the exumbrellar pathway as does MIC, since the extract failed to initiate any potentials in that tissue. Presumably the extract excites crumpling through subumbrellar rather than exumbrellar conducting systems. Second, pointing is activated during SIIC but not during MIC therefore. SIIC involves an additional conducting pathway, called the pointing pathway by Mackie and shown by him to be neuronal¹. There is some evidence that a fourth conducting pathways is involved in SIIC. Pointing cannot occur in MIC since all four radial muscles contract tetanically in both the subumbrella and peduncle. In SIIC however, the radial muscles in the peduncle do not contract tetanically (note the difference in the peduncular extensions in Fig. 1*b* and *c*) and are free to be used for pointing; therefore, two functionally distinct regions of the radial muscles (subumbrellar and peduncular) are postulated. It is possible that the bridge pathway¹, a conducting system of yet unknown function, may play a role in selectively activating these separate portions of the radial muscles.

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Bivalent-cation-stimulated ATPase activity at preformed exocytosis sites in *Paramecium* coincides with membrane-intercalated particle aggregates

TRICHOCYSTS of *Paramecium* cells, although representing morphologically specialised¹ secretory vesicles, obey the general rules for exocytosis. Expulsion can be triggered by artificial increase of the intracellular Ca^{2+} -concentration^{2,3} ($[\text{Ca}^{2+}]_i$), that is, by Ca^{2+} -mediated stimulus-secretion-coupling^{4,5}; the trichocyst and the cell membrane fuse to form a transient exocytosis canal^{6,7} through which the protein-contents⁸ are discharged. Unlike other secretory

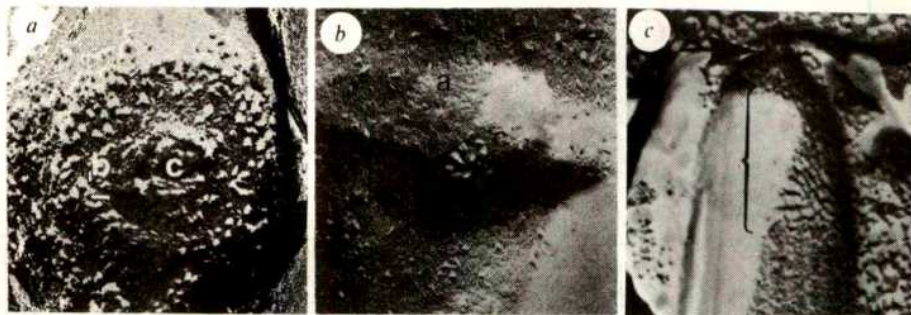
granules, however, trichocysts are, long before discharge, closely attached in a regular pattern to the plasmalemma which, at these attachment sites, contains regular arrays of membrane-intercalated particles^{9,10} (MIP) (Fig. 1*a,b*). Many MIP occur also within the tip region of the trichocyst membrane; Fig. 1*c* also shows some non-etchable 'membrane-connecting material' between trichocyst and cell membrane which probably corresponds to electron-dense materials seen on ultrathin sections⁹. 'Central granule patches' would correspond to 'fusion rosettes' described also for other ciliates¹⁰ and actinopods^{11,12}. The sporadic occurrence of similar MIP aggregates at presumable exocytosis sites of endothelial¹³ and neurohypophysis¹⁴ cells might indicate that *Paramecium* cells are capable of maintaining an otherwise rather ephemeral situation, that is, the stage of membrane-to-membrane attachment preceding membrane fusion. The requirement of energy¹⁵ and Ca^{2+} (refs 4, 5) and the presumable involvement of bivalent-cation-stimulated ATPase activity¹⁶ in the course of exocytosis led us to search for a functional correlate of the specialised structures observed at exocytosis sites.

Paramecium aurelia K 401, was cultivated monoxenically (*Enterobacter agglomerans*) in a standardised Difco medium. For cytochemistry, conducted at 20 °C, cells were washed three times with a ~300-times excess of 10mM Tris-HCl pH 7.0. Washed cells (~ 2×10^4 per 0.2 ml) were fixed in suspension for 60 s with doubly distilled 2.5% glutaraldehyde in 0.1 M cacodylate buffer pH 7.0 and diluted 1:10 with buffer for 14 min. This method was developed to overcome the marked sensitivity of ATPases to fixation and the discharge of trichocysts by too low fixative concentrations¹⁷. After washing (3×5 min) in 10 ml 0.1 M Tris-maleate buffer pH 7.0 cells were incubated for 1 h in the same buffer (eventually at pH 5 or 9) with the following additives: 2.4 mM Pb^{2+} as a capture ion, 5 mM substrates (ATP as Tris salt), 5 mM bivalent and/or 20 mM monovalent cations (chlorides); inhibitors (5 mM) were added also to the last wash before incubation. All chemicals were of the highest purity available and dissolved immediately before incubation. After three washes cells were osmicated (1% OsO_4 in cacodylate buffer pH 7.0, 1 h) (except cells for X-ray microanalysis), dehydrated in acetone and embedded in Durcupan ACM (Fluka). Uncontrasted silver sections were examined in a JEM 100C electron microscope (100 kV). $[\text{K}^+, \text{Na}^+, \text{Ca}^{2+}, \text{Mg}^{2+}]_o$ were determined by atomic absorption photometry (Beckman, model 1248), flame photometry (Instrumentation Lab. model IL 343) and colorimetry in spectral photometers.

Reaction product (RP) was found around the sites where trichocysts are attached to the plasmalemma (Fig. 2) in the following conditions (pH 7): with $\text{Pb}^{2+} + \text{ATP} + \text{Ca}^{2+}$ or Ba^{2+} ; some, but apparently less, RP was also formed with Mg^{2+} . Addition of Mg^{2+} or Na^+ and/or K^+ did not change the reaction obtained with Ca^{2+} . Similarly RP was formed (although independent from the addition of mono- or bivalent cations) with *p*-nitrophenylphosphate, acetylphosphate, Na-AMP and less with Na- β -glycerophosphate as substrates. RP formation was always independent from osmication or section staining. RP was absent at pH 5 and moderate at pH 9 in conditions yielding strong RP at pH 7.

In the following controls no RP was formed (pH 7): omission of Pb^{2+} or chelation by a fourfold excess of 1,2-dihydroxybenzene-disulphonic-(3,5)Na₂-salt; omission of appropriate substrates; omission of bivalent cations in presence of Tris-ATP; with Na₂-ADP ($\pm \text{Ca}^{2+}$), Na₂-GTP ($\pm \text{Ca}^{2+}$), adenylyl imidodiphosphate (a substrate for the cytochemical assay of adenylate cyclase¹⁸), 3',5'-cyclic AMP or its dibutyl derivative as tentative substrates; after heat exposure during fixation (5 min, 80 °C). Maintaining the original fixative concentration also results in lack of RP. When cells were fixed, soaked in phosphate

Fig. 1 Freeze-cleave replicas obtained with a Balzers BAF 300 (turbomolecular pump; 10^{-6} mbar; 2 min etching at -100°C ; Pt- and C-evaporation by electron beam evaporators). Shadowing direction is from bottom to top. ($\times 72,000$.) a, Cytoplasmic half of the plasmalemma at a trichocyst attachment site; a, 300-nm large double ring⁶ of MIP; b, intermediate MIP; c, 'central granule patch' of MIP⁶ equivalent to 'fusion rosette'¹⁰. b, Outer half of the plasmalemma



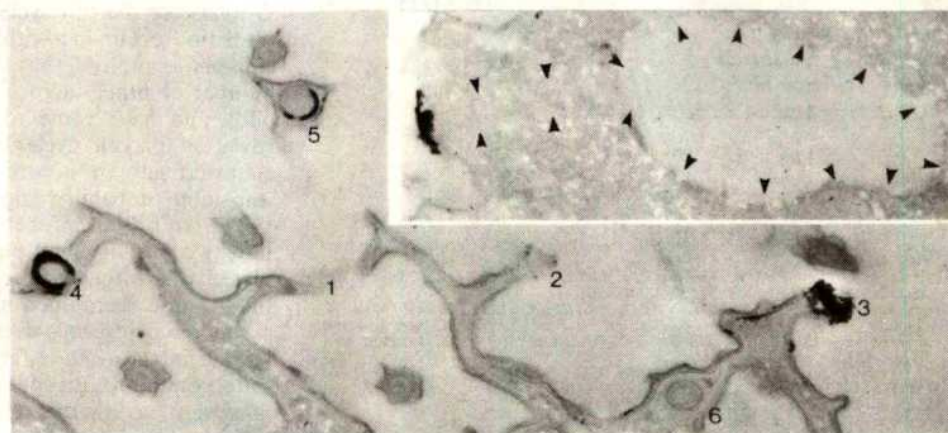
of a trichocyst attachment site; explanations for a, c as in Fig. 1a. On this fracture plane the 'ring' is represented by pits instead of MIP, whereas about 10 'rosette' MIP (c) become better visible on this fracture plane. c, Inner half of a trichocyst membrane; note 'annuli'²⁵ of MIP (bracket); corresponding to 'e-type structures' in ref. 6, followed by impressions from the microtubule collar⁶ in the lower portion and non-etchable 'membrane-connecting material' (arrow) between the trichocyst proper and the plasmalemma. For comments on possible structural equivalents of ATPase activity see text.

buffer pH 7 and then in 2.4 mM Pb^{2+} , electron-dense deposits were scattered randomly. Sr^{2+} at pH 9 with subsequent substitution by Pb^{2+} , a method designed for K^{+} , Na^{+} -ATPase¹⁹, gave only spurious RP with various substrate-cation combinations, which resulted in RP formation under 'standard conditions' at pH 7.

At pH 7 media containing Pb^{2+} + Tris-ATP + Ca^{2+} or Pb^{2+} + *p*-nitrophenylphosphate were used with various inhibitors: ouabain for Mg^{2+} -dependent Na^{+} , K^{+} -stimulated 'transport' ATPase^{20,21}, L-tetramisole for alkaline phosphatase²², and atractyloside for transmembrane nucleotide transport²³. These inhibitors all failed to reduce RP formation. Considerable blockage was obtained with Salycarn or *N*-ethylmaleimide which both inhibit various ATPases^{20,23} by interaction with SH-groups.

The ionic milieu can be characterised as follows: $[\text{K}^{+}]$, $[\text{Na}^{+}]$, $[\text{Ca}^{2+}]$, $[\text{Mg}^{2+}]$ were constantly around 1.8, 0.4, 0.17 and 0.17 mM respectively in the culture medium, 0.05, 0.2, 0.05 and 0.04 before glutaraldehyde fixation and 0.04, 0.04, 0.04 and 0.06 during incubation (without salts added). In living paramecia $[\text{K}^{+}]$ ≈ 20 mM (ref. 24), whereas $[\text{Ca}^{2+}]$ probably equilibrates around 0.03–0.05 mM (ref. 25) for our low $[\text{Ca}^{2+}]$ values. Assuming that permeability changes due to glutaraldehyde fixation were similar to those in other cells²⁶, we expect from extrapolation a maximum of $[\text{K}^{+}]$ < 10 mM and $[\text{Ca}^{2+}]$ ≈ 0.04 mM; if the membrane potential generated by high $[\text{K}^{+}]$ and $[\text{Ca}^{2+}]$ in paramecia²⁴, were to break down in response to the insult of chemical fixation, $[\text{K}^{+}]$ and $[\text{Ca}^{2+}]$ could be around 0.04 mM at the sites of RP formation (unless when added in excess). In conclusion, with the possible exception of K^{+} , the concentration of the relevant cations can be assumed to be < 0.1 mM, if no salts were added.

Fig. 2 Cytochemical demonstration of ATPase activity obtained with 5 mM Ca^{2+} + 5 mM Tris-ATP + 2.4 mM Pb^{2+} , pH 7, in conditions specified in the text. This 'grazing' section contains several attachment sites of trichocysts (1–6) in a regular pattern (compare with ref. 6). Only where the section contains uppermost tip regions—with structures represented in Fig. 1—reaction product (RP) is deposited. (1–6) Designate a sequence proceeding from the outside to the inside; (1, 2, 6) are unreactive because the sectioning level is still too high (1, 2) or too low (6). On positive sites RP fills the space delineated by its surrounding membranes (see text and ref. 6). Inset: Longitudinal section through a trichocyst; outlines are marked by arrows; RP only in tip region. Unstained sections ($\times 31,500$).



To determine whether bivalent cations (5 mM) are truly stimulative we analysed whether Pb^{2+} is the true capture ion in our experiments, since the presence of bivalent cations during glutaraldehyde fixation also results in formation of RP-like deposits on some biomembranes²⁷, including Ca^{2+} -binding sites of paramecia^{3,28,29}. Since X-ray microanalysis revealed the simultaneous presence of P and Ca in such deposits, rapid redistribution of intracellular ATP occurring on fixation²⁸ could lead to cleavage and PO_4^{3-} -capturing by bivalent cations at sites of persisting ATPase activity^{3,27,28}, even if these cations were not stimulative. But, X-ray microanalysis of RP formed with Pb^{2+} + Tris-ATP + bivalent cations revealed the selective enrichment of P and Pb only (Fig. 3). Hence, Ca^{2+} , Ba^{2+} and (to a lesser degree) Mg^{2+} are truly stimulative and Na^{+} , K^{+} non-stimulative ions.

The hydrolysis of various orthophosphate substrates in the absence of additional ions (for K^{+} see above) might be due to a phosphatase which splits phosphorylated intermediates generated by various transport ATPases^{20,21}, including Ca^{2+} -, Mg^{2+} -ATPases^{30,31}. Thus the RP at attachment zones of trichocysts to the plasmalemma probably represents an ATPase stimulated by bivalent cations—in physiological conditions most probably a Ca^{2+} -(transport)-ATPase.

Various transport ATPases, including Ca^{2+} -ATPase³² are membrane-integrated oligomeric proteins which would show up in freeze-cleaved membranes as MIP of the usual size range³³. Absence of RP from potential attachment sites (unpublished observations) which contain only 'rings' of MIP if not occupied by trichocysts^{2,34} points mainly towards 'central granule patches' (fusion rosettes) and possibly 'membrane-connecting material' and/or 'annuli' as

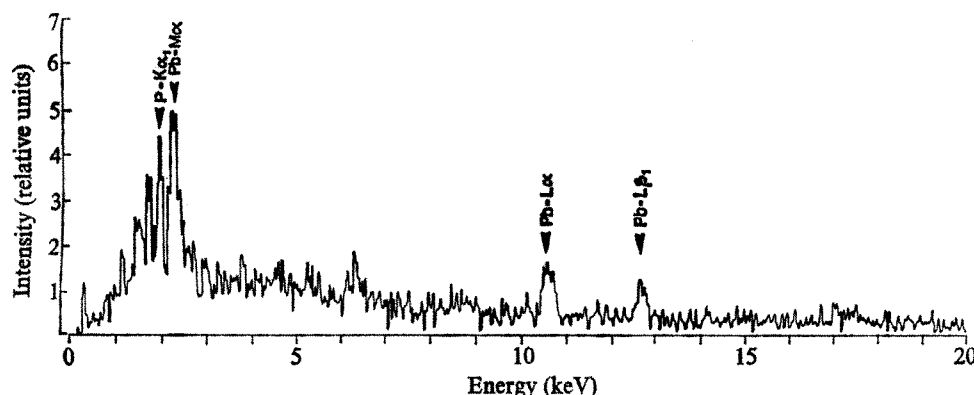


Fig. 3 Energy spectrogram obtained from reaction product, specified in Fig. 2, by X-ray microanalysis using an analytical electron microscope JEM 100C (60 kV) in the scanning transmission mode and combined with an eucentric goniometer stage (40°), a Kevex detector and a Link spectrometer unit, system 290. Experimental conditions were as before^{3,30} but C-coated nylon-grids (Touzart-Matignon) were used to mount ~1000-Å thick, non-osmicated, unstained sections. Note the occurrence of Pb-lines Ma (2.342 keV), La (10.550), Lβ₁ (12.612) and of P-Kα₁ (2.013), while Ca-Kα (3.690) is absent. Si-Kα (1.739 keV) is an artefact.

candidates for the structural equivalent(s) of bivalent-cations-stimulated ATPase. Since RP might diffuse within the small cleft between apposed membranes (plasmalemma, trichocyst and alveolar membrane, see ref. 6), further investigations have to envisage a discrimination among these possible candidates.

Several possibilities have to be considered to explain how Ca²⁺ and ATP could intervene in the final steps of exocytosis (see also ref. 16). The local restriction of RP indicates a mechanism involving local [Ca²⁺]-regulation at exocytosis sites. This could entail (1) regulation of membrane repulsion or attraction depending on charge screening, or (2) conformational changes of the 'rosette' MIP or of 'membrane connecting material' reminding the postulated mechanocomplex¹⁰ and the actomyosin-like fibrillar materials occurring at exocytosis sites¹⁵; furthermore (3) Ca²⁺-dependent regulation of lipid phase transitions³⁵ and intramembraneous segregation phenomena¹⁰ could be involved in the local MIP clustering described.

Trichocyst discharge is a very effective, specialised form of exocytosis, but the structural-functional correlations involved could clarify the final steps of exocytosis in other systems.

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Transition probability and the origin of variation in the cell cycle

It is well established that the cell cycle times of cells cultured *in vitro* are highly variable although the origin of this variability has remained unclear^{1,2}. A number of theories have been advanced to explain the variation in cell cycle times although none of them give entirely satisfactory fit to the experimental data³. Any proposal as to the origin of this variability must take account of the observation that most of the variability occurs in the G₁ phase of the cell cycle and that it is the length and variability of this phase which is the major determinant of the length and variability of cell cycle times within a cell population¹. A new model of the cell cycle has been proposed independently by two groups that suggests that variability in the G₁ phase of the cell cycle arises as a result of a probabilistic transition occurring within G₁ (refs 3, 4). Using time-lapse cine-photography I have examined the possible role of a number of other factors which might determine this variability and have come to the conclusion that the observed variation in cell cycle times *in vitro* is qualitatively and quantitatively in accord with variation predicted by the transition probability model of the cell cycle⁴.

The transition probability model divides the cell cycle into two parts—the B phase (containing S, G₂, M and part of G₁) where the cells are proceeding towards division, and the A state (contained in G₁) where the cells are awaiting a random triggering event before proceeding. A cell may remain in the A state for any length of time, but in steady-state conditions the probability (the transition probability) of a cell leaving the A state and entering the B phase in any unit of time is constant. In a population of cells the shortest intermitotic time is shown by a cell that leaves the A state immediately it enters. Intermitotic times greater

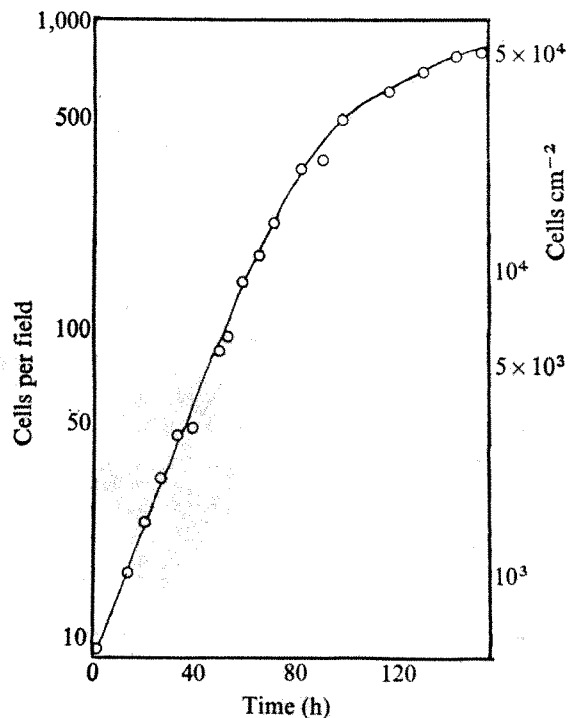


Fig. 1 Growth curve of Swiss 3T3 cells. The cells were plated in Dulbecco's modified Eagle's medium containing 10% foetal calf serum. The cultures were filmed using a 4-min interval and cell counts made from projections of individual frames of the film. The saturation density of these cells is about 5×10^4 cells cm^{-2} .

than this minimum (equal to the duration of B phase, T_B) should be exponentially distributed in a population of cells with identical T_B values and identical transition probability. A graph of the logarithm of the percentage of undivided cells (α_t) against time (the α curve) would show two

components, since the value of α_t will be 100% for times up to T_B and then decay exponentially. In practice the α curves often exhibit some initial curvature that has been ascribed to variation of T_B within a cell population⁴. But, the values of T_B in sibling cells are apparently very similar so that the distribution of differences of intermitotic times of sister cells (the β plot) is also exponential and parallel to the exponential tail of the α curve^{5,6}.

Heterogeneity of cell cycle times approximating to an exponential frequency distribution may be generated in ways other than assuming a random transition. Since most of the observations to date have been on uncloned cell populations, the observed variability could arise from pooling data from cells with differing constitution. To investigate this possibility the behaviour of single clones of the established Swiss 3T3 mouse fibroblast cell line was observed. Cells were plated at low density and filmed until the culture reached confluence (Fig. 1), and α and β curves were constructed generation by generation for eight separate clones for up to six generations (Fig. 2). Subsequent generations were not monitored because cultures were no longer in a steady state over the period of a generation and the cells were increasingly hard to follow so no further data was collected. The α and β curves reveal two types of clonal behaviour. Most of the clones (six of eight) behaved like the clone (clone 5) illustrated in Fig. 2a. The first four generations of this clone were pooled to provide enough data for α and β curves; it made little difference if the early generations were pooled within or between clones (not shown). After some initial curvature the α curves became exponential and closely parallel to the β curve providing evidence that the variation in cell cycle times is not due to clonal heterogeneity. For this clone the minimum value of T_B was 11.6 h in the first four generations, 13 h by the fifth and 14.3 h by the sixth. Similar increases in the value of T_B with successive generations were noted for all the clones examined although T_B varied

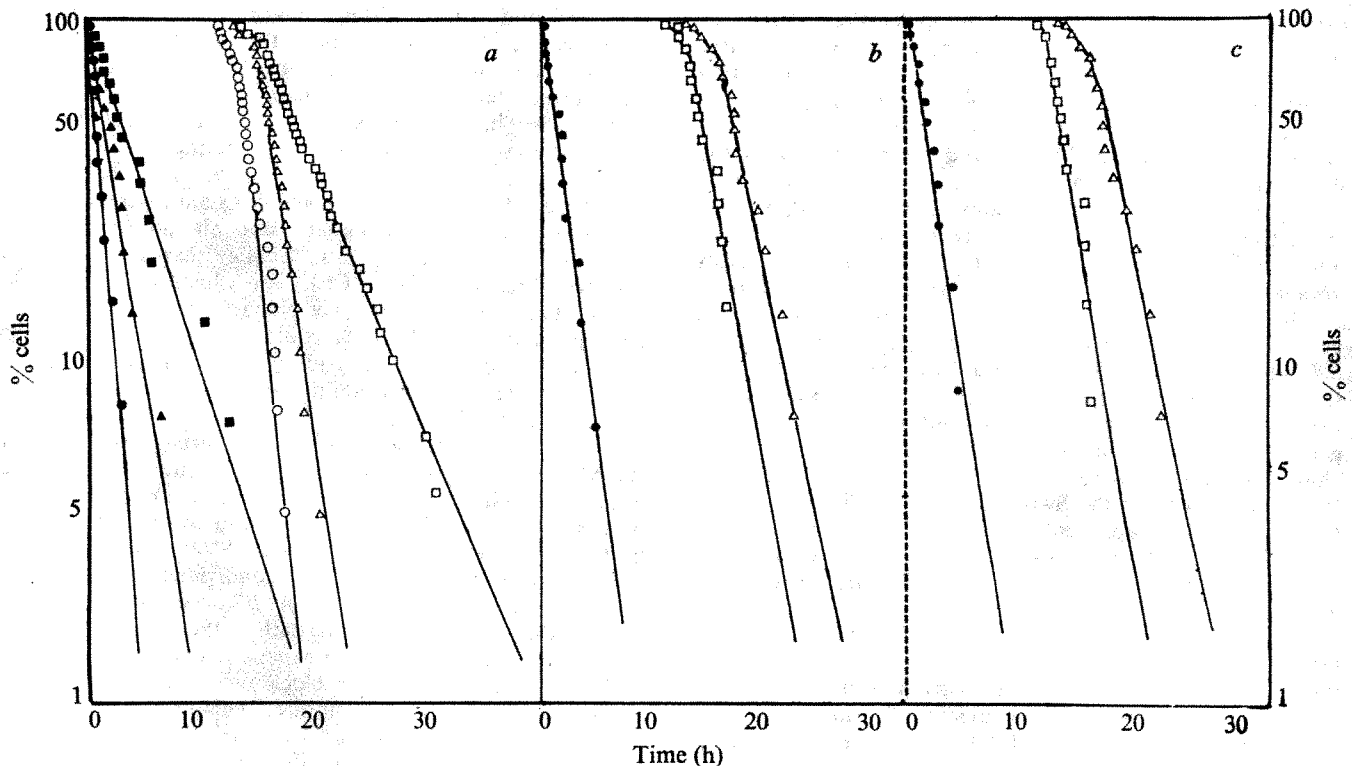


Fig. 2 Semilogarithmic plot (open symbols) of the % of cells (α_t) undivided against age (t) for a clone in the experiment shown in Fig. 1. Also shown (closed symbols) are plots of the % of sibling pairs (β_t) with a difference of intermitotic times $> t$. *a*, $\circ$, $\bullet$, Pooled first four generations; $\triangle$, $\blacktriangle$, fifth generation; $\square$, $\blacksquare$, sixth generation (clone 5). *b*, α plots, β plots of the pooled first four generations of another clone (clone 3) from the experiment shown in Fig. 1. $\square$, $\triangle$, Descendants of the two progeny of the first division. The β curves of these two subclones were identical (not shown) and have been pooled for clarity. *c*, As (*b*) but for the fifth generation. The β curve is pooled from both subclones.

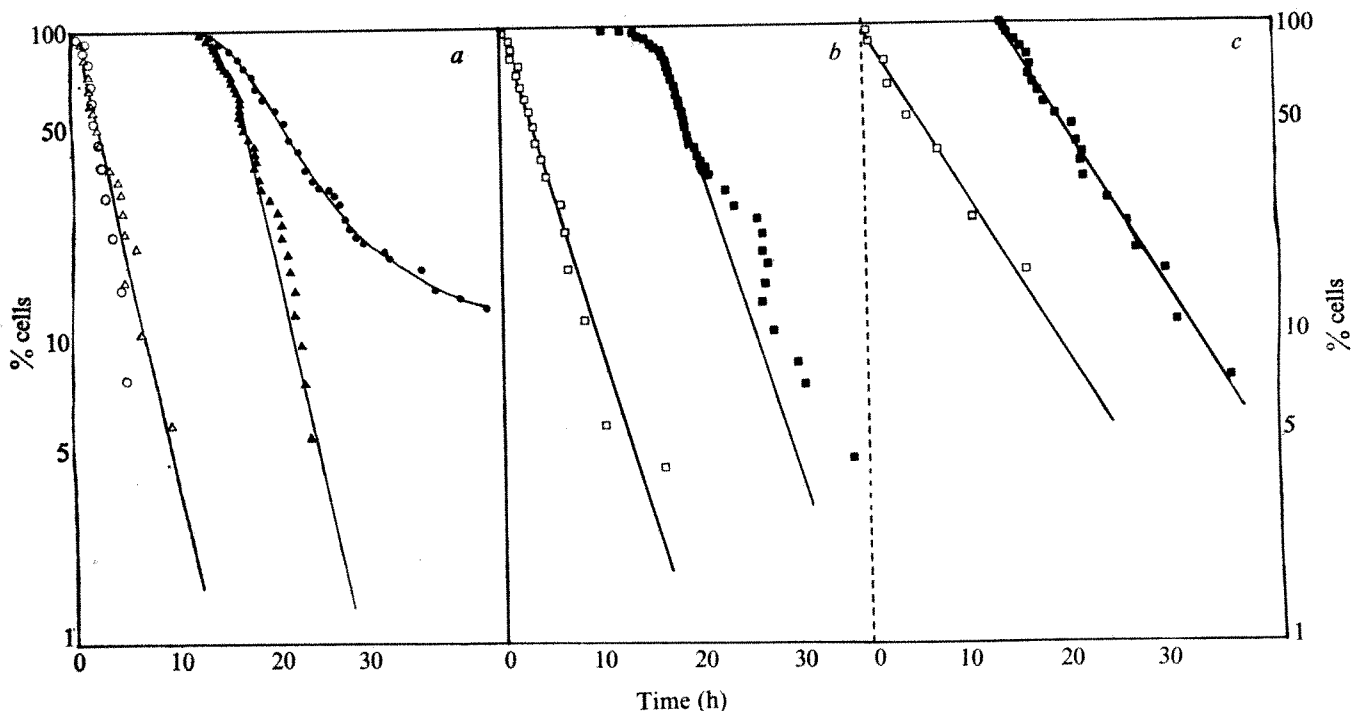


Fig. 3 α and β plots of primary mouse fibroblast (MEF) and primary chicken fibroblasts (CEF). Mouse embryo cells were prepared from whole embryos of Tylers original mice. Cells were plated at 2×10^5 per 5-cm dish in Dulbecco's modified Eagle's medium containing 10% foetal calf serum. Chicken fibroblasts were prepared from 10-d embryos of C/O brown leghorn chicks. Cells were plated at 4×10^5 per 5-cm dish in Dulbecco's modified Eagle's medium supplemented with 10% Tryptose phosphate broth, 1% foetal calf serum and 1% heat-inactivated chicken serum. Both cultures were filmed using a 4-min interval. *a*, α and β curves for MEF. Intermittent times were collected from the progeny of several different cells; $\bullet$, pooled data excluding the three clones shown separately; $\circ$, corresponding β curve. Δ , α Curve from pooled data of three clones in the MEF population; $\triangle$, corresponding β curve for pooled data of three clones. *b*, *c*, α Curves (closed symbols) and β curves (open symbols) for the progeny of two separate CEF cells. In (*b*) the deviation of the points from the drawn α curves is probably due to extensive T_B heterogeneity within a subfraction of the population. Nevertheless, the intermitotic times of 60% of the cells lie on the drawn α curve but the β curve derived from all the cells of the clone is linear.

between the clones and between the generations. Nonetheless, a major cause of the lengthened cell cycle in later generations is the decreased value of the transition probability (estimated from the slope of the β plots).

The other type of clonal behaviour (two out of eight clones) is illustrated in Fig. 2*b* and *c*. In these cases the descendants of the daughters of the first division behave as separate clones differing mainly in the length of T_B . As a consequence the α curves are parallel to each other and the β curves superimposable, this behaviour persists until at least the fifth generation. After this the lifespans of the sixth generation subclones no longer overlapped and the transition probability in the two subclones was different so the T_B difference could not be calculated (not shown). The results from clones of this type show that T_B may (at least in part) be heritable and persists over several generations. This generation of T_B heterogeneity within a clone may be the source of T_B variation within a cell population. Results from the Swiss 3T3 cell line do not support the idea that the apparent exponential component of the variability in cell cycle times within a population arises from clonal heterogeneity as this variability arises even within clones.

It might be argued that karyotypic instability in established cell lines accounts for the variation in cell cycle times even within a cell clone. This possibility has been explored by examining the behaviour of primary cultures of mouse and chicken fibroblasts which are presumably isogenic and diploid. The behaviour of mouse embryo fibroblasts (MEF) is depicted in Fig. 3*a*. The α curve for the pooled data from many different cells (excluding three clones, see below) is sigmoid indicating that a significant fraction of the population did not divide. Nevertheless, a β plot from the same cells is exponential as non-dividing

cells had non-dividing sisters thus giving no value for the difference in intermitotic times. The descendants of some cells could be followed for a number of generations, data from three clones were pooled and α and β plots shown in Fig. 3*a*. The β curve is exponential and parallel to the exponential tail of the α curve. Furthermore, the β curve of the clones is superimposable on the β curve of the pooled data from all the cells which excluded these clones, indicating that the clones are not an unrepresentative fraction of the viable cells. Similar experiments with primary chick fibroblasts (CEF) are illustrated in Fig. 3*b* and *c*. There was considerable clonal heterogeneity in the CEF population so the data could not be pooled. Results from two clones (Fig. 3*b* and *c*) show differences in T_B as well as transition probability, in both cases exponential β curves were obtained.

The α curves shown in this paper rarely extend below the 5% level of undivided cells. This is due to the limited number of cells that can be analysed in a single generation of a single clone. Pooled data from many generations of BALB/c3T3 cells transformed with SV40 virus extended these α plots down to the 0.3% undivided level and the β curve down to the 1% level. The β curve was exponential and parallel to the exponential tail of the α curve⁶.

It could be argued that the exponential kinetics observed is due to escape from some block unwittingly imposed by the conditions of *in vitro* culture. This seems unlikely as free-living organisms such as *Euglena* also have exponentially distributed cell cycle times⁷ as do *Escherichia coli* with cycle times as short as 30 min which are highly unlikely to be blocked (L. Martin, personal communication). Furthermore, the shape of frequency of labelled mitosis curves obtained *in vivo* may be accurately predicted from transition probability theory^{3,4,8-10}, which suggests that cells

in vivo may undergo a similar type of random transition to that observed *in vitro*.

The results in this paper demonstrate that the variability in cell cycle times *in vitro* is not entirely due to pooling data from many cells with different pedigrees nor does it arise from an artefact of using cell lines. These results, together with the fact that the β curves are exponential (which is necessary and a sufficient condition for the existence of a random transition in the cell cycle (R. S. and J. A. Smith, unpublished)), support the idea that the variation in cell cycle times *in vitro* is due in part to a random transition in the cell cycle and that transition probability is a key function that regulates cell proliferation.

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Hybrid cell lines with T-cell characteristics

THE hybridisation of established cell lines with differentiated cells provides a useful strategy for the production of cell lines having differentiated properties and unlimited growth potential. Clones of these hybrids could be particularly useful in the dissection of cellular interactions of the immune system, a network involving numerous specific cell types which display characteristic ensembles of surface and internal markers. We describe, here, the production and partial characterisation of hybrid cell lines with surface antigens characteristic of T lymphocytes.

Köhler and Milstein^{1,2} have shown that the fusion of mouse myeloma cells cultivated *in vitro*, with spleen cells from immunised mice, gives rise to B-cell hybrid cell lines which secrete monoclonal immunoglobulins with antibody activity to the immunising antigen. Although 30–40% of the spleen cells used for fusion were T cells, none of the hybrids expressed the Thy-1 surface antigen, a characteristic marker for mouse T cells. Without expression of this (or some other) T-cell marker, it is difficult to determine in retrospect whether the non-producing hybrids were T cells-myeloma fusion products in which Thy-1 is repressed.³

In contrast with results from the myeloma fusion studies, we found that fusion of spleen cells with a mouse T-lymphoma cell line (BW 5147) gives rise to hybrid cell lines which express T-cell and not B-cell markers. Hybrid populations were obtained by fusing spleen cells from (BALB/c×SJL)_F₁ mice with HAT-sensitive BW 5147-AzG^R, Ou^R cells (kindly provided by Dr Robert Hyman). The BW 5147 cells were HGPRTase negative and resistant to 1×10^{-3} M ouabain. Spleen donors were immunised by intraperitoneal injection of 100 µg of DNP-KLH on alum with 2×10^7 *Bordetella pertussis*, and boosted 3 months later by intravenous injection of 20 µg of DNP-KLH. Cells were harvested from the spleens 4 days later. Hybrids were also obtained by fusing BW 5147 with spleen cells from non-immunised CBA and AKR donors which had been stimu-

lated for 4 or 5 d in mixed lymphocyte cultures (MLC) by irradiated allogeneic spleen cells.

For the fusion, a mixture of 10^8 spleen or MLC-derived cells and 10^7 BW 5147 cells in Dulbecco's Modified Eagles medium plus 10% foetal calf serum (DME-FCS) was pelleted at 400g. The medium was removed and fusion initiated by the gradual addition of 2 ml of 50% (w/w) polyethylene glycol (PEG) 1,000 or 1,500 in serum-free DME. After 2 min at 37 °C, the suspension was then gradually diluted to 50 ml (over the course of 10 min) with serum-free DME and then pelleted by centrifugation⁴.

The pelleted cells were next resuspended and cultured in 0.2- or 2-ml volumes at $1-2 \times 10^6$ cells ml⁻¹ in selective medium (DME-FCS, 100 µM hypoxanthine, 10 µM aminopterin, 30 µM thymidine and 3.3×10^{-4} ouabain). Preliminary experiments have shown that neither spleen cells nor BW 5147 cells survive for longer than a few days in this medium; therefore, after 14 d, selective medium was removed and replaced with DME-FCS. All populations surviving after 2 weeks were presumed to be hybrids since survival requires that the gene for HGPRTase from the spleen cell parent and the (genetic) information for continuous growth from the lymphoma parent both be present in each cell.

In later experiments, cell numbers per well were adjusted to allow one or a few hybrid clones to be seeded. Hybrid populations were transferred when adequate growth was observed. Clonal populations were obtained either by limiting dilution in 0.25-ml wells or by soft agar cloning. Only the (BALB/c×SJL)_F₁ hybrid populations were cloned at the time of this report.

The putative hybrids were analysed for DNA content per cell using flow micro fluorometry in a fluorescence activated cell sorter (FACS-II, Becton-Dickinson Electronics Laboratory, Mountain View, California) after fluorescent straining of the DNA with propidium iodide in hypotonic (0.1%) sodium citrate⁵. Approximately 40 hybrid populations were tested within the first 2 weeks after hybridisation. All had G₁ DNA contents which were about the sum of the DNA contents of spleen cell (diploid mouse) and the near tetraploid lymphoma used for hybridisation.

The expression of both parental Thy-1 alleles on 29/39 hybrid cell populations (and clones) obtained from hybridisation of Thy-1 differing cells demonstrates directly that the hybrids arose from fusion of normal spleen cells and the BW 5147 lymphoma (see Table 1). Specificity of typing was confirmed by quantitatively absorbing the anti Thy-1.2 with hybrid cells and then testing the absorbed antiserum on normal thymocytes to demonstrate removal of anti Thy-1.2 activity. The demonstration that hybrids of BW 5147 with a Thy-1.1 carrying strain, AKR, express only Thy-1.1 rules out expression of a cryptic Thy-1.2 allele by the hybrids (Table 1).

Identification of the splenic lymphocyte subpopulation which fused with BW 5147 to give rise to hybrids expressing both parental Thy-1 alleles is difficult in retrospect; however the increased frequency of such hybrids obtained by fusion of MLC cells, which are nearly all T cells, suggests that the Thy-1.1, 1.2 hybrids are probably derived from fusions of normal T cells with the lymphoma (Table 1). Whether the fusions which gave rise to hybrids expressing only the BW 5147 Thy-1.1 allele were also T cell-lymphoma fusions may become clear as the hybrids are tested for more T-cell markers. Interestingly, the spleen cell hybridisation gave rise to two clones which express neither parental Thy-1 allele, that is in which the BW 5147 Thy-1.1 is lost or repressed.

Preliminary tests for Ly 1,2,3, and Ia markers on the hybrids are consistent with hybrids being derived from fusions of normal T cells and lymphoma cells. The BW 5147 lymphoma comes from a strain which carries

Table 1 Expression of Thy-1 antigens on hybrid cells

Experiment	Hybridisation	Parental populations	Number of hybrid populations expressing Thy-1 alleles*			
			1.1+1.2	1.1	1.2	—
921	AKR T Lymphoma BW 5147 (Thy-1.1)	× (BALB/c × SJL)F ₁ Spleen (Thy-1.2)	18	7	0	2
C.2	AKR T Lymphoma BW 5147 (Thy-1.1)	× CBA Spleen MLC† (Thy-1.2)	11	1	0	0
A.2	AKR T Lymphoma BW 5147 (Thy-1.1)	× AKR Spleen MLC (Thy-1.1)	0	13	0	0
Kohler and Milstein ⁴	BALB/c Myeloma MOPC 21 (X63) (Thy-1 negative)	× BALB/c Spleen (Thy-1.2)	0	0	0	>10

*Populations from experiment 921 are individual clones, populations from experiments C.2 and A.2 are isolates from different fusion hybrids and may contain one or more clones. Thy-1 phenotype was determined by direct cytotoxicity using congenic anti-Thy 1 antisera (experiment 921) and non-congenic antisera (experiments C.2 and A.2). Thy-1 phenotype of several clones in experiment 921 was confirmed by quantitative absorption studies.

†Cells from a 5-d mixed lymphocyte culture (MLC) of spleen cells stimulated with irradiated allogenic cells. Nearly all surviving cells in this culture are T cells from the responder spleen.

Ly-1.2 and Ly-2.1; however it expresses neither of these characteristic T-cell markers. T cells from (BALB/c × SJL)F₁ mice carry Ly-1.2 and Ly-2.2 determinants. Some of the clones derived from the (BALB/c × SJL)F₁ hybridisation selectively express Ly-1.2 determinants, some selectively express Ly-2.2 determinants while the majority expresses neither. Similarly, Ia determinants selectively expressed on a very small subpopulation of lymphocytes (most likely T) in Ia^a haplotype mice and not on BW 5147 (Ia^k) are expressed in some of the hybrid clones derived from the (BALB/c × SJL)F₁ Ia^d/Ia^a haplotype spleen cells. Among normal T lymphocytes, selective expression of Ly and Ia determinants defines supopulations responsible for the various T-lymphocyte functions^{6,7}.

From the above, it is clear that the hybridisation of spleen cells with an appropriate established cell line (such as the BW 5147 T lymphoma) can yield continuous cell populations expressing characteristic T-cell surface markers. The preliminary evidence for expression of functional T-cell subset markers by these hybrid populations encourages us to examine them further for the presence of T-cell functions such as helper, suppressor and cytotoxic activity. It is apparent that the expression of any of these functions by clonal populations of continuous cell lines would greatly facilitate the analysis of these T cell-mediated functions.

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The adjuvant active fraction of delipidated mycobacteria

FREUND'S complete adjuvant (FCA) has been used to influence both cell-mediated and humoral immune responses. Mycobacteria are the essential constituents of FCA and different active fractions have been extracted from mycobacteria of various strains¹⁻⁶. The purpose of the work reported here was to try to answer the following question: are cell-mediated and humoral immune responses related to the stimulatory effect of different bacterial components or to the adjuvant activity of a same bacterial fraction? Data presented here suggest that the stimulation of these two kinds of immunological response is related to the adjuvant activity of the same bacterial fraction, which has been identified as the lipoglycopeptide fraction extracted from mycobacterial delipidated cells (LG). Furthermore, the study of the relationship between adjuvant activity and chemical structure showed that LG is comprised of two distinct moieties: a glycopeptide moiety with cellular activity and a lipid moiety stimulating humoral immune response.

LG was isolated, by selective solubilisations, from bacterial products of the human 'Peurois' strain of *Mycobacterium tuberculosis* (Pasteur Institute, Paris) according to the procedure previously described⁷. Cleavage by alkaline hydrolysis⁸ of the ester linkage of LG resulted in two moieties: (1) a liposoluble moiety (37%) mainly composed of unsaturated fatty acids (mycolic acids) as determined by elementary analysis, solubility tests and comparison with the infrared spectrum of an authentic mycolic acid specimen; and (2) a hydrosoluble moiety (63%) identified by paper chromatography and infrared spectrometry, as a glycopeptide. This glycopeptide has the following characteristics: approximate molecular weight of 27,000 (determined by analytical ultracentrifugation); $[\alpha]_D^{20} = 36^\circ$; $C = 0.1$; neutral sugars: 94-98% arabinose and galactose in an approximate molar ratio of 1.6:2; amino acids and amino sugar: 2-6% glycine, alanine, glutamic acid, diaminopimelic acid and galactosamine. Catalytic hydrogenation⁹ of LG resulted in a derivative in which only unsaturated mycolic acids of the lipid moiety were saturated, called lipid moiety modified

derivative (LMD) or glycopeptide moiety non-modified derivative (GND) and acetylation of LG resulted in a derivative in which only free hydroxyl groups of the glycopeptide moiety were acetylated, called glycopeptide moiety modified derivative (GMD) or lipid moiety non-modified derivative (LND).

When LG was submitted to both catalytic hydrogenation and acetylation, a two-moieties-modified derivative was obtained, called LGMD (lipid and glycopeptide moieties modified derivative).

Female albino guinea pigs (400–500 g) were sensitised by injection into each hind footpad of 0.1 ml of a water-in-oil emulsion consisting of 1 part paraffin oil containing the adjuvant fraction, 2 parts Tween 80 and 5 parts saline containing the protein antigen, crystalline ovalbumin (OA). On day 21 after sensitisation, the animals were skin tested by intradermal injection of various amounts of OA in 0.1 ml saline into the clipped flank and the reactions were read 24 and 48 h later.

BD F₁ (DBA/2×C57BL/6) mice weighing approximately 20 g were each injected intraperitoneally with 10⁸ sheep red blood cells (SRBC) after about 15 min, various amounts of adjuvants in 0.1 ml of the water-in-oil emulsion were given by intraperitoneal injection. After 4 d, the animals were killed and the number of spleen cells forming or releasing antibody to SRBC was determined by the haemolytic plaque assay of Jerne and Nordin¹⁰, without modification.

Figure 1 illustrates the influence of LG and LND on the average number of plaque-forming cells (PFC) in mice 4 d after immunisation with SRBC. No positive reaction was observed in animals which had received LMD or LGMD as adjuvants.

Table 1 shows that inclusion of LG or GND in the antigen injection resulted in strong skin reactivity of delayed type hypersensitivity to the test antigen. No visible skin reaction was observed in animals sensitised with GMD or LGMD.

The results reported here show that in FCA, LG can substitute for whole mycobacterial cells. LG comprises two moieties: a hydrosoluble glycopeptide moiety and a liposoluble lipid moiety. The glycopeptide moiety is responsible

Fig. 1 Influence of various amounts of LND, FCA and LG on the average number of direct (IgM) plaque-forming cells (PFC) in mice 4 d after immunisation with sheep red blood cells (SRBC). LG, lipoglycopeptide; LND, lipid moiety non-modified derivative; FCA, Freund's complete adjuvant; LMD, lipid moiety modified derivative; LGMD, lipid and glycopeptide moieties modified derivative.

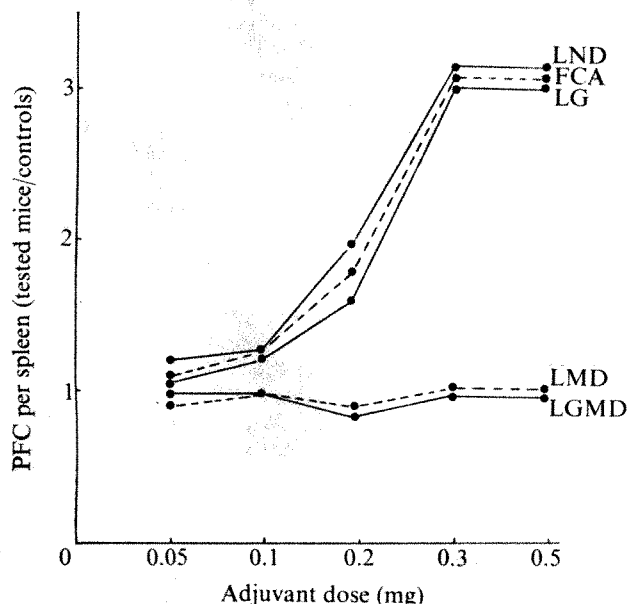


Table 1 Mean diameter of redness in guinea pigs skin at sites of injection of various amounts of test antigen

No. of animals in groups	Sensitising antigen (1 mg per animal)	Sensitisation Adjuvant incorporated in antigen (0.4 mg per animal)	Delayed skin reactions		
			Test antigen dose (mg per 0.1 ml)		
			0.1 mg	3 µg	0.2 µg
6	OA*	FCA	20 (N)†	9	6.5
6	OA*	LG	19 (N)	7	3
6	OA*	GND	20.7 (N)	12	7
6	OA*	GMD	0	0	0
6	OA*	LGMD	0	0	0
6	OA*	None	0	0	0

*Crystalline ovalbumin.

†Mean diameter of redness (mm) at 48 h; N, central necrosis.

FCA, Freund complete adjuvant; GND, glycopeptide moiety non-modified derivative; GMD, glycopeptide moiety modified derivative; LGMD, lipid and glycopeptide moieties modified derivative.

for the cell-mediated response, and the humoral immune response is attributed to the lipid moiety. The ability of LG to act as an adjuvant of both cell-mediated and humoral immune responses seems to be related to some state of balance between these two moieties, since (1) a decrease in hydrosolubility of the glycopeptide moiety by acylation of free hydroxyl groups destroyed cellular activity of LG; (2) an increase in liposolubility of the lipid moiety by catalytic hydrogenation inhibited humoral immune response; and (3) the derivative LGMD in which both lipid and glycopeptide moieties were modified, was found to be completely inactive as an adjuvant for either cell-mediated or humoral immune responses.

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IgM-IgG switch-over among antibody-forming cells in the mouse

DIFFERENTIATION of immunocytes from stem cells to antibody secreting B cells involves sequential expression of genes coding for heavy chain constant regions. This differentiation seems to be independent of thymus activity and antigen stimulation, for nude mice¹ and 15-week human foetuses² as well as germ-free mice³ and piglets⁴ have proportions of surface IgM, IgG or IgA bearing cells similar to those found in normal adults. It has therefore been suggested that the switch-over from IgM to IgG and IgA expression occurs before antigen contact which leads to differentiation from small B lymphocytes to plasma cells⁵. But during antibody production after antigenic stimulation a small proportion (1–3%) of mouse plaque-forming cells (PFC) secrete both IgM and IgG (refs 6, 7) and clones of cells derived from a single antigen-stimulated precursor secrete both IgM and IgG antibody⁸. These observations indicate that the IgM→IgG switch could be antigen driven. We have investigated the two alternatives by culturing

individual PFC from mice immunised with sheep erythrocytes (SRBC) to study whether daughter PFC produce the same or a different Ig class. We have found that a part of IgM producing parental PFC can generate 'in vitro' IgG

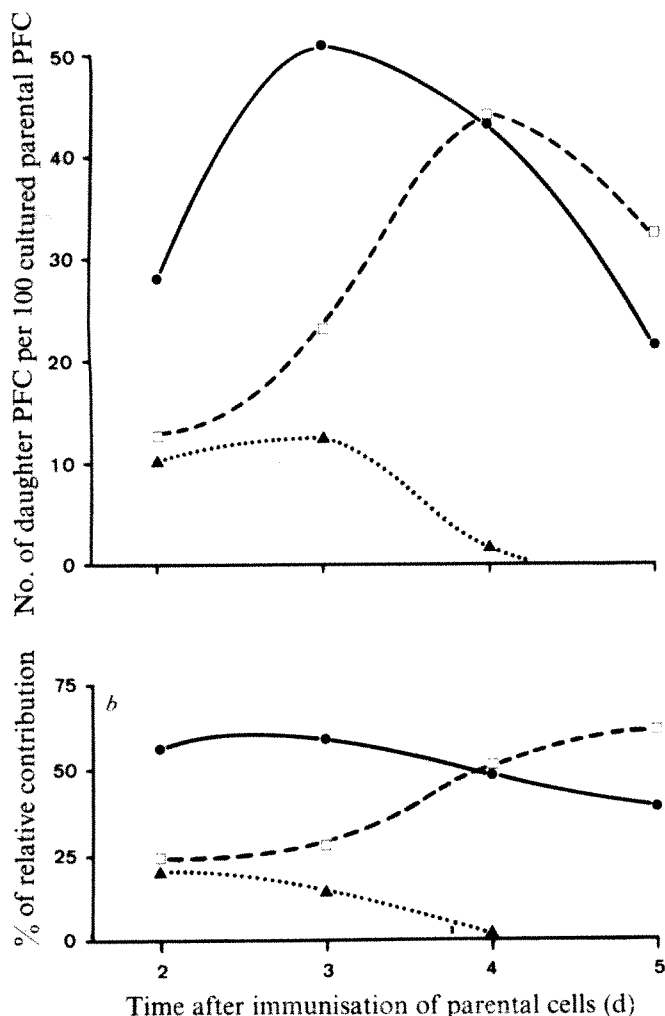


Fig. 1 Generation of daughter PFC producing either IgM or IgG from individually cultured parental PFC taken on the indicated days after immunisation ●, Parental IgM PFC generating IgM daughters; □, parental IgG generating IgG daughters; ▲, parental IgM generating IgG daughters. *a*, The numbers of daughter PFC are recorded per 100 individually cultured parental PFC (standardised % from 96 to 186 parental PFC at each point). *b*, Relative contribution of various daughter PFC in the total amount of daughter PFC in each day after immunisation. Spleen cells from Swiss mice given SRBC (4×10^8) on day 0 were taken on days 2, 3, 4 or 5 and placed in non-shaken mass cultures as described previously⁹ for selecting the most resistant PFC in 'in vitro' conditions. These cultures were supplemented with LPS at a final concentration of $7.5 \mu\text{g ml}^{-1}$. The cells were taken 24 h later, mixed with SRBC and complement, distributed in 5- μl microdroplets covered with paraffin oil and incubated for 45 min at 37°C . Individual PFC (parental PFC) were micro-manipulated out of the droplets and transferred into separate wells of polyacrylamide plates^{10, 11}. These wells had each been preseeded with about 1,000 heavily irradiated (2,500 rad) normal Swiss mouse spleen cells and 1,000 SRBC. The whole plate was sunk into plastic Petri dishes (Falcon) containing medium made half with the medium taken from the preseeded culture and half with fresh medium. The plates were maintained for 24 h in cell culture conditions and the content of each well was spread together with indicator SRBC and complement on antibody slides (Bellco) incubated for 1 h at 37°C and examined for haemolytic plaque formation (daughter PFC). For investigating the Ig class switch over from the parental to daughter PFC, the parental and daughter PFC were detected either directly (IgM producers) or indirectly using an enhancing serum a rabbit anti-mouse IgG serum mixed with a goat anti-mouse- μ -chain serum for revealing only IgG producing PFC. Control wells containing irradiated cells and SRBC as well as old and fresh culture fluids but not micromanipulated PFC were left in each experimental plate. In a total of over 350 such wells not all PFC were detected.

producing daughters, but this switch occurs only during the first 3 d after immunisation.

Adult mice were immunised with SRBC, their spleens were taken in the following days and cultured for 24 h in mass-cultures and then the PFC were micromanipulated and individually cultured for the subsequent 24 h in the conditions described in Fig. 1. The cultures contained LPS (*Escherichia coli* 055B5 lipopolysaccharide, Difco) for mitogenic stimulation in absence of T-cell helper activity in the individual PFC cultures. Three series of experiments were undertaken: in the first IgM producing (direct) parental PFC appearing in the mass-cultures were individually cultured and then plated with the direct procedure for revealing IgM producing daughter PFC; in the second series, the parental and daughter PFC were revealed with the indirect procedure using an enhancing an anti-mouse IgG serum mixed with an anti-mouse μ -chain serum (Table 1); in the third series, again IgM producing parental PFC were individually cultured but the daughter PFC were revealed by the indirect method for detecting only IgG producers so as to study the IgM $\rightarrow$ IgG switch from parental to daughter PFC.

In this type of experiment not all individually cultured PFC yield a progeny. In fact, only 10–20% of parental PFC generate daughter PFC (refs 11, 13). But, the percentage of parental PFC yielding daughter PFC and especially the number of the latter (1–5 per parental PFC), can give a representative figure of the proliferative potential of each category of parental PFC. As shown in Fig. 1*a* day 3 parental IgM PFC generated the higher number of IgM daughters (51%) and thereafter their yield decreased whereas the 4th day IgG parents produced the higher number of IgG daughters (44%). The generation of IgG producing daughter cells from day 2 and day 3 parental IgM producers was rather low (10% and 12.5% respectively) and sharply decreased thereafter (1.5% from day 4 and 0% from day 5 parental IgM PFC). It was, however, significant because the relative contribution of IgG daughters of IgM PFC seems quite considerable, about the half on day 2 and the third on day 3 in the total number of IgG producing daughters of IgG and IgM parental PFC (Fig. 1*b*). On the other hand, it seems that not all IgM PFC can generate IgG daughters: 21% of day 3 parental IgM PFC (39 out of 186) produced IgM daughters (total, 95; 51%) whereas only 8.7% (14 out of 160) of day 3 parental IgM PFC generated IgG daughters (total 20; 12.5%). This is in agreement with observation that a proportion of lymphocytes bear surface IgM only whereas others express both IgM and IgG or IgM and IgA before expressing the latter alone¹. The low numbers of IgG daughters of IgM PFC detected may be due to the generation of both IgM and IgG daughters from these IgM parental PFC. It is also likely that these parental PFC already began to synthesise low quantities of IgG. These possibilities could not be tested using our methods because parental and daughter PFC endowed with high rate synthesis and secretion of Ig could only be revealed by either the direct or the indirect method.

To what extent do the above findings correspond to the 'in vivo' events? To answer this question we compared the relative frequency of IgM and IgG producing daughter PFC with that of IgM and IgG PFC found in 'in vivo' studies. Wortis *et al.*¹⁴ observed that on the 3rd day after immunisation of mice with SRBC, 47% of PFC produced IgM and 53% IgG and on the 5th day 38% and 62% respectively. Sell *et al.*¹⁵ did not find significant numbers of IgG PFC on day 3 whereas on day 5 they found 42% IgM PFC and 58% IgG PFC. Compared with these figures we recorded 59% IgM and 41% IgG daughters of day 3 parental PFC and 39% and 61% respectively from day 5 parental PFC (Fig. 1*b*). Therefore it could be inferred that this sampling of daughter PFC is not very different from that found 'in vivo' at correspond-

Table 1 Numbers of haemolytic PFCpcr 10^6 spleen cells taken on day 2 or 5 after immunisation of mice with SRBC

Days after immunisation	Direct PFC	Anti- μ chain 1 : 200	PFC enhanced with Anti-IgG 1 : 3, 200*	Anti- μ 1 : 200+ anti-IgG 1 : 3, 200†
2	98	2	122	6
	48	1	57	2
	36	0	43	3
	60	2	78	4
5	796	3	1,120	496
	687	0	2,374	415
	835	2	1,608	1,083
	1,078	5	2,530	885

*Rabbit anti-mouse IgG serum (Pasteur Institute, Paris) exhibiting its maximum enhancing activity at the dilution of 1.3,200.

†Goat anti-mouse IgM serum (Meloy) made monospecific anti- μ -chain by absorption with mouse IgG in the laboratory and giving in immunoelectrophoresis a single arc of precipitation corresponding to mouse IgM (ref. 12).

ing intervals after immunisation with SRBC. This parallelism does not exclude the possibility that the IgM→IgG switch described here is induced by LPS present in the cultures since it has been shown the LPS alone accelerate expression of surface and cytoplasmic IgG¹⁶. Secretion of IgG 'in vitro' does not appear until 2 weeks after onset of LPS stimulated mouse spleen cell cultures¹⁷, however.

The considerable frequency of IgG PFC daughters of day 2 IgM PFC (44% of total IgG daughters, Fig. 1b) may mean that on day 0 of primary stimulation only IgM precursors are triggered by antigen. The more so as treatment of cells with anti-mouse μ -chain serum when culturing them¹⁸ or transferring them into irradiated recipients¹⁹ results in inhibition of IgM as well of IgG and IgA antibody production. Cells already committed to IgG could also be triggered by antigen during primary (Fig. 1) or during secondary response²⁰, however and generate progeny of IgG producing cells. It is therefore possible that even during the primary response a part of IgG producers are derived from IgG precursors.

Lawton *et al.*⁵ studying the development of Ig class diversity concluded that the switch from IgM to IgG expression occurs before antigen contact. Our results showing that early after antigen stimulation a part of IgM PFC generate IgG producers suggest that the cells are sensitive to antigen before the full accomplishment of the commitment to one particular Ig class production. The process of sequential expression of genes coding for C_H could, however, be independent of the antigen-driven differentiation from B lymphocytes to plasma cells. Indeed findings that germ-free mice³ and piglets⁴ have normal adult proportions of cells bearing μ , γ or α surface Ig as well as present evidence that on days 4 and 5 after immunisation no IgM→IgG switch occurs in spite of the existence of many proliferating IgM PFC and the presence of antigen (Fig. 1) strongly suggest that IgM→IgG switch is rather directed by an internal genetic control. It may in fact be that B cells become sensitive to antigen as early as Ig (IgM) molecules appear on their surface, the switch-over to IgG or IgA production taking place whether or not antigenic stimulation intervenes.

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Simple model for self-non-self-discrimination in invertebrates

THE immune mechanisms which enable invertebrates to eliminate infectious agents are not well understood. There have been many reports, however, that invertebrates produce 'recognition factors' which interact with foreign substances and subsequently aid the phagocytosis of these substances^{1–5}. Such recognition factors have been isolated from worms, molluscs, arthropods, echinoderms and protochordates, and have been shown to have agglutinating and bactericidal activities as well as opsonic properties. But, unlike the vertebrate immune system, there is little evidence for a memory component in the production of these factors^{3–5}. The recognition factors are usually large molecules which can be readily dissociated into subunits and which in no way resemble the immunoglobulins present in vertebrates^{3–12}. They apparently have specificity for carbohydrate structures and, in many cases, an individual has several distinct subpopulations of recognition factors which bind different sugar specificities^{3–12}. The recognition factors seem to be synthesised by haemocytes in the haemolymph^{13,14}. After secretion they can, in many invertebrate species, bind to the surface of haemocytes as well as persist in a soluble form in the haemolymph^{3–5,14–18}. Both the soluble and cell-bound forms of the recognition factors can augment the phagocytosis of foreign substances. Thus, the invertebrates have evolved a simple recognition system which enables them to discriminate between 'self' and 'non-self'. In this paper I propose that the recognition factors are composed of some of the glycosyl transferases which the invertebrate uses to synthesise its own carbohydrate side chains. Such a model guarantees the maintenance of rigid self non-self discrimination.

The model is presented schematically in Fig. 1 and would particularly apply to the advanced invertebrates. It is arbitrarily proposed that the invertebrate synthesises an oligosaccharide side chain of five sugar residues. Each sugar residue is attached by a different glycosyl transferase which has specificity for the sugar acceptor¹⁹. Thus, five proteins (transferases) with different sugar binding properties are

present in the organism. These five transferases act as the subunits of the recognition factors, are secreted by haemocytes into the haemolymph, and randomly associate into hexamers.

The recognition factors do not react against self as the self sugars they recognise have been blocked by glycosylation. This important point is made clearer if the mode of action of glycosyl transferases is considered (Fig. 2). These enzymes catalyse the transfer of a monosaccharide from a sugar nucleotide to the terminus of a specific sugar acceptor¹⁹. Figure 2 depicts the enzyme-substrate reaction of glycosyl transferase 4 in Fig. 1. It can be seen that transferase 4 interacts with terminal monosaccharide 3 and catalyses the attachment of monosaccharide 4. Of course, once glycosylation has occurred, monosaccharide 3 is blocked from further interaction with transferase 4. Thus, the glycosyl transferases which make up the recognition factors are unable to bind to self as the terminal sugars they recognise have been masked by glycosylation. In addition, it should be noted that the recognition factors would probably only bind foreign substances and not glycosylate them (as in Fig. 2) as it is most unlikely that sugar nucleotides exist extracellularly in the haemolymph.

In the model presented in Fig. 2, it is proposed that polymerisation of the transferases into a recognition factor is initiated by an additional protein (no. 6) which has an acceptor site on the haemocyte surface. The inclusion of this additional protein imparts cytophilic and opsonic properties to the recognition factors. In order to prevent inter-

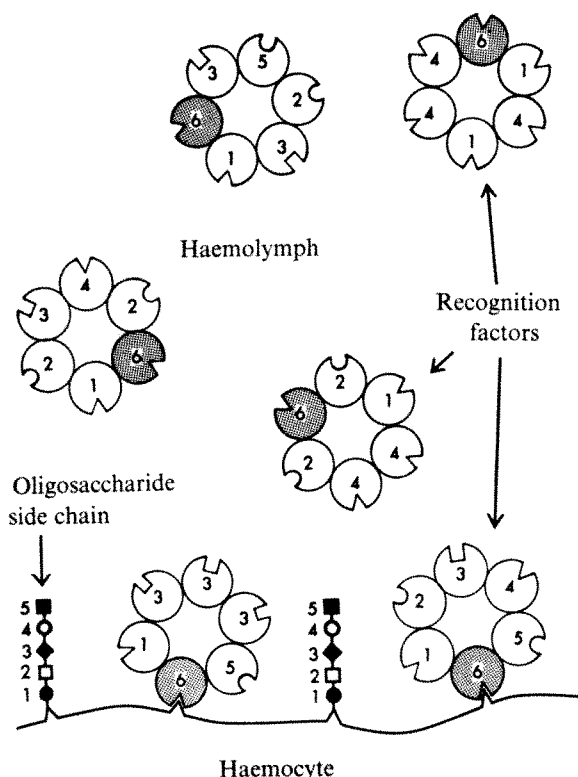
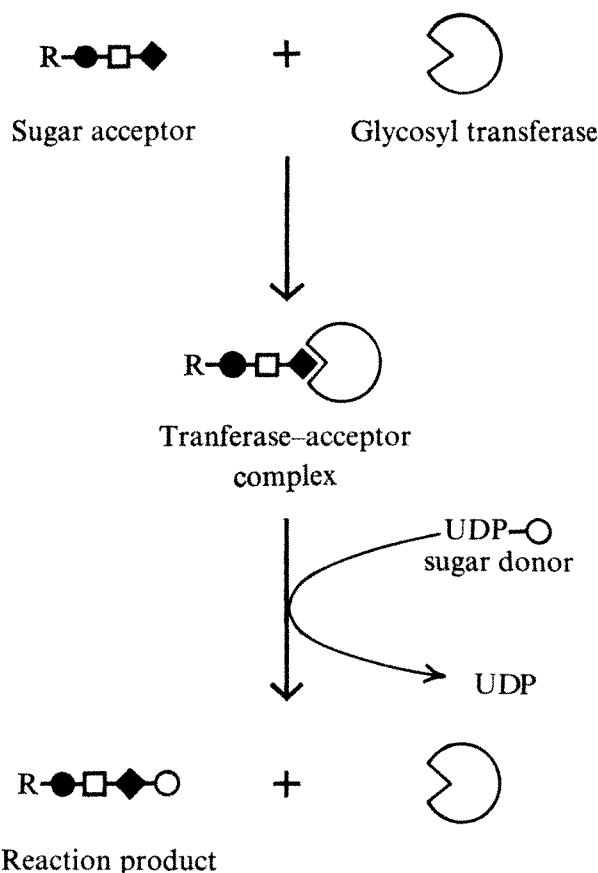


Fig. 1 A possible model for the recognition factors in invertebrates which augment the phagocytosis of foreign substances. Various monosaccharides on the haemocyte surface, which constitute an oligosaccharide side chain, are represented by circles, diamonds and squares and are numbered 1-5. Each monosaccharide is inserted in the oligosaccharide by a specific glycosyl transferase (proteins numbered 1-5) as depicted in Fig. 2. Thus, transferase 2 binds to monosaccharide 1 and catalyses the attachment of monosaccharide 2, transferase 3 binds to monosaccharide 2 and facilitates the attachment of monosaccharide 3, and so on. The transferases randomly associate into recognition factors (hexamers), this polymerisation being initiated by an additional protein (no. 6). Protein 6 has an acceptor site on the haemocyte surface which enables the recognition factors to be cytophilic for haemocytes. For details of the model see text.



Reaction product

Fig. 2 Mode of action of glycosyl transferases. A trisaccharide sugar acceptor is depicted, linked to a lipid or protein carrier (R). The circles, diamonds and squares represent the different monosaccharides. The glycosyl transferase specifically binds to the terminal monosaccharide (◆) of the sugar acceptor and catalyses the transfer of a monosaccharide (○) from its uridine diphosphate (UDP) donor to the terminus of the acceptor. The resultant reaction product is a tetrasaccharide. This diagram depicts the enzyme-substrate reaction of glycosyl transferase (no. 4) in Fig. 1, that is, the transferase which binds to monosaccharide 3 and catalyses the attachment of monosaccharide 4.

action (and therefore autoaggression) between haemocytes, only one cytophilic subunit can be included in each hexamer.

Although there are only five basic recognition units in this model, the fact that they polymerise in a random manner into hexamers, enables them to react in a multi-point fashion with foreign substances. This greatly increases the effective specificity range of the recognition factors as it is well established that multipoint interactions have a much higher binding efficiency than single point interactions²⁰.

This model of immune recognition has several important theoretical advantages. First, it ensures that self-reactive recognition factors are not generated. Second, due to multi-point binding, a comparatively wide range of foreign antigenic specificities can be recognised. Third, the system can withstand a high degree of genetic polymorphism without self-reactivity appearing. Fourth, if any infectious agent produces hydrolytic enzymes which degrade the cell surface carbohydrates of the invertebrates host, the subterminal sugars are unmasked and thus these damaged and infected cells can be readily recognised and encapsulated by the host's haemocytes.

The model presented in Fig. 1, however, could vary in several ways. Obviously, the variety of glycosyl transferases and the number of subunits incorporated into the recognition factors could vary considerably between invertebrate species. Furthermore, the cytophilic subunit may be absent in some invertebrates and, in these cases, the factors may eliminate infectious organisms by merely acting

as lytic and neutralising agents. Similarly, only some of the glycosyl transferases produced by the invertebrate may be secreted and incorporated into recognition structures. Conversely, not all the glycosyl transferases appearing in the recognition factors may be able to act as glycosylating enzymes. For example, if a mutant form of enzyme 3 arose (see Fig. 1) which had lost its glycosylating activity, then enzymes 4 and 5 would be unable to construct a sugar side chain, even though their enzymatic properties were still intact.

There would be no memory component in this form of immune recognition. It is conceivable, however, that infectious agents may activate the haemocytes to multiply and secrete the recognition factors more rapidly. There is, in fact, some evidence in invertebrates that foreign substances can induce the production of recognition factors^{3,5,21,22}. Whether the recognition factors described above mediate the specific transplantation immunity observed in some invertebrate species remains to be determined^{3,5,23,24}. The existence of specific short-term memory in these transplant responses suggests, however, that another recognition system, possibly the forerunner of vertebrate T-cell immunoglobulin, may be involved²⁵.

In conclusion, it should be noted that there is a mounting body of evidence which suggests that, in both plants and animals, glycosyl transferases have a role in cell-cell recognition and cell-glycoprotein interactions^{19,26,27}. These findings add credence to the possibility that glycosyl transferases could have developed into a primitive protective system against infectious agents. In fact, some of the sugar binding proteins detected in vertebrates, such as the galactose-binding proteins in liver which mediate the clearance of desialysed plasma proteins^{20,28,29}, may represent a remnant of the invertebrate recognition system described.

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Succinyl bee venom melittin is a leukocyte chemotactic factor

A NUMBER of leukocyte chemotactic factors of defined structure have been described. They include formyl-methionyl tripeptides (f-Met-Leu-Phe and related peptides) which attract neutrophils at concentrations as low as 10^{10} – 10^{11} M (refs 1 and 2), the eosinophilotactic tetrapeptides Val-Gly-Sur-Glu and Ala-Gly-Ser-Glu (ECF-A) (ref. 3) and

HETE; a 12-hydroxy-unsaturated fatty acid derivative of arachidonic acid⁴. All of these have in common a net negative charge and a hydrophobic moiety. The availability of chemotactically active molecules of known structure now allows structure-activity relationships to be examined, and preliminary evidence that f-Met peptides bind to a receptor on the leukocyte surface has been presented^{5,6}. Leukocytes respond to a great many chemotactic factors of diverse structure and, in order to understand fully the binding sites involved, it will be useful to have as many defined chemotactic factors for study as possible. This paper reports the chemotactic activity of the succinylated form of the bee venom peptide, melittin.

Figure 1 shows the structure of melittin⁷. It is a cationic, amphipathic peptide in which residues 1–20 are predominantly hydrophobic and residues 21–26 hydrophilic. Melittin is surface-active and has been shown to disrupt cell membranes and artificial phospholipid liposomes^{8,9}. Unmodified melittin is lytic for neutrophil leukocytes at concentrations of 10^{-6} M and above⁸, and I was unable to detect any chemotactic activity for unmodified melittin at any concentration. Habermann and Krowallek¹⁰ showed that the cytolytic effects of melittin could be abolished if the α - and ϵ -amino groups were blocked by succinylation, thus reducing the positive charge. Since succinylation would yield a product with broadly similar physicochemical characteristics to the chemotactic factors listed above, it might be predicted that succinyl-melittin (S-melittin) would have chemotactic activity for leukocytes.

Gly – Ile – Gly – Ala – Val – Leu – Lys – Val – Leu – Thr –
Thr – Gly – Leu – Pro – Ala – Leu – Ile – Ser – Trp – Ile –
Lys – Arg – Lys – Arg – GluNH₂ – Glu(NH₂)₂

Fig. 1 Structure of melittin.

Figure 2 shows that a pure preparation of S-melittin attracts human blood neutrophil leukocytes into micropore filters in a dose-dependent manner. To demonstrate that the cells were responding to a gradient of S-melittin and thus that S-melittin determined the direction of their locomotion (chemotaxis) and not merely their rate of locomotion (chemokinesis), the activity of S-melittin was examined in a checkerboard assay, a procedure introduced by Zigmond and Hirsch¹¹ to distinguish between these two reactions.

Table 1 shows that the leukocytes responded to both the absolute concentration of S-melittin and to the concentration gradient, thus showing both reactions. Assuming that melittin exists as a tetramer⁷, its effective concentration was between 5×10^{-5} M and 5×10^{-6} M. Only heavily succinylated preparations of melittin were sufficiently non-cytotoxic to show chemotactic effects and at least 80% of free amino groups required to be blocked to obtain a chemotactically active peptide.

Melittin contains neither methionine nor phenylalanine, and thus does not contain any sequence similar to those of the f-Met peptides which are active as chemotactic factors. If these peptides act by binding to a stereospecific cell surface receptor, it is unlikely that S-melittin binds to the same receptor. It is more probable that the leukocyte surface contains more than one site at which chemotactic reactions can be initiated. The effective chemotactic concentration of S-melittin was lower (ED_{50} about 5×10^{-6} to 10^{-5} M) than that of f-Met tripeptides suggesting a lower binding affinity. The locomotor response induced by the ED_{50} was, however, comparable in both cases.

Since melittin is known to bind to lipid bilayers both in cells and in liposomes, it is suggested that this binding, which could be to a saturable binding site but is not likely

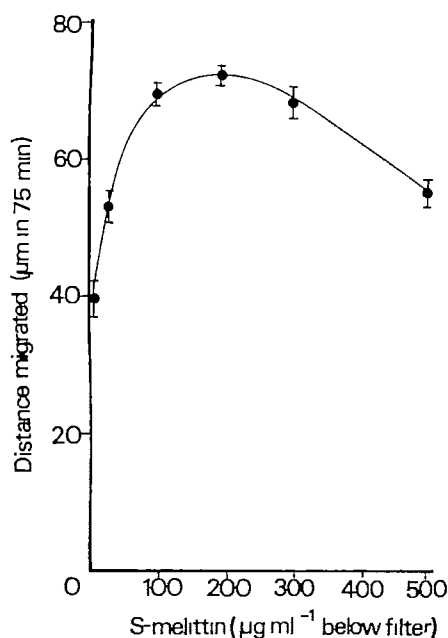


Fig. 2 Migration of human blood neutrophils into micropore filters (3-μm poresize, Millipore) towards S-melittin at various concentrations below the filter. Cells were prepared in Gey's solution with human serum albumin (1 mg ml⁻¹) and the chemotaxis assay was carried out as described previously¹³ using the leading-front method¹¹ to measure migration. Bee venom melittin was a highly purified product obtained from Dr Barbara Banks, University College, London, UK. It was treated with succinic anhydride (35 mg per 20 mg peptide) as described by Habermann and Krowallek¹⁰. The ninhydrin reaction¹⁴ was used to determine free amino groups before and after succinylation. The preparation used in the experiment shown had 83% of its free amino groups blocked.

to be to a stereospecific receptor, is an adequate signal for initiation of chemotactic reactions. I have presented a similar hypothesis to explain the chemotactic properties of denatured proteins and proteins with diverse covalently bonded non-polar groups¹². It now seems likely that chemotactic reactions can be initiated by signals from molecules binding to the cell surface at a variety of sites and with a variety of affinities. This versatility seems reasonable in view of the wide diversity of molecules now shown to initiate such reactions¹³. The charge of surface-active chemotactic factors such as S-melittin is obviously important as well as their hydrophobicity, since blocking the amino

Table 1 Migration of human blood neutrophils in various absolute concentrations and concentration gradients of S-melittin

S-melittin μg ml ⁻¹	Migration (μm into 3-μm poresize filter in 75 min)				
	0	5	95	185	275
0	52				
5		54	82(62)	89(70)	102(65)
95			58(63)	71	95(73)
185				68(73)	75
275					57(78) 76(81) 91

Figures on the diagonal from upper left to lower right show migration in increasing absolute concentrations of S-melittin but in the absence of a gradient. Above the diagonal, cells were moving in a positive gradient, below it in a negative one. The figures without brackets show the migration actually observed, those within brackets show the migration expected if the cells had been responding to absolute concentration alone (calculated after Zigmond and Hirsch¹¹). Note that cells migrated further in positive gradients and less far in negative gradients than would be expected if they had responded only to the absolute concentration of the attractant suggesting that there was a chemotactic response to the S-melittin gradient.

groups of melittin with negatively charged succinyl groups yields a product which is chemotactic at concentrations at which the unconjugated, cationic, analogue damages the cell.

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Theophylline-induced changes in ion transport and conductance in human small intestinal mucosa

THE central role of cyclic 3',5'-adenosine monophosphate in intestinal secretion has been emphasised recently^{1,2}. Electrolyte absorption is converted to secretion^{3,4} and mucosal conductance is reduced^{5,6} by increases in epithelial cyclic AMP concentrations and this is held to mediate the profound secretion provoked by cholera and other forms of infective 'toxigenic' diarrhoea. The mechanisms of the intestinal response to cyclic AMP are incompletely understood and we therefore examined this response in human intestinal mucosa. We report here that changes in mucosal permeability are largely determined by the state of the lateral intercellular spaces. When these spaces are open, permeability is increased by cyclic AMP and when they are closed, permeability is decreased. These changes in permeability have a profound effect on the ion transport responses to cyclic AMP.

In ileal mucosa theophylline induced a rapid rise in electrical potential difference (p.d.) and short circuit current (s.c.c.) persisting for at least 40 min (Fig. 1). Total ionic conductance, calculated from p.d. and s.c.c., fell by about 25%, an effect similar to that observed in rabbit ileum⁶.

This conductance fall has been attributed to effects on paracellular shunt pathways and one possible anatomical counterpart is the elimination of lateral intercellular spaces which follows cell swelling induced by theophylline⁶. This high conductance pathway normally contributes about 80% of total epithelial conductance⁶, and closure of the channels could clearly influence total conductance. If this hypothesis is correct, then opening the spaces should prevent this response to theophylline. We therefore re-examined the influence of theophylline in the presence of glucose (15 mM) which dilated the lateral spaces due to enhanced fluid absorption (Fig. 2). Here, instead of rising, the mucosal resistance, the reciprocal of conductance, fell from 37 to 21 Ω cm⁻² (Fig. 1). 3-O-methyl glucose also caused theophylline to lower ileal resistance from 41 to 26 Ω cm⁻² and 35 to 28 Ω cm⁻² in two studies. Since 3-O-methyl glucose is transported but not metabolised, this response is not a non-

specific metabolic effect of glucose. It also occurred with glucose on the mucosal, but not with it present solely on the serosal side of the tissue, confirming an association with transport. Glucose alone did not influence resistance, but did so when added to tissues pretreated with theophylline (Fig. 1).

The simplest explanation for these findings is that dilatation of lateral spaces revealed a theophylline-induced increase in conductance. This would bring intestinal mucosa into line with high resistance epithelia such as toad bladder, in which theophylline enhances conductance¹⁰. Theophylline thus apparently has two effects—decreasing conductance due to cell swelling and closure of lateral spaces, and increasing it when the lateral spaces are opened by glucose. Similar changes were found in rat ileum by Walling and Kimberg (personal communication), but others have not demonstrated this effect in rabbit ileum exposed to cholera toxin¹¹. This discrepancy may be due to species differences, but is otherwise unexplained.

There is some conflict about the mechanisms for the

intestinal secretion. Nellans *et al.*¹² demonstrated that theophylline inhibits a coupled Na-Cl influx at the luminal cell membrane while Powell *et al.*¹³ presented convincing evidence for the stimulation of a coupled Na-Cl secretion in rabbit ileum. Yet others have postulated that theophylline stimulates a rheogenic secretion of Cl, uncoupled from Na (ref. 14). Since mucosal conductance influences electrolyte transport we examined the effect of theophylline on ion

Fig. 2 Electron micrograph of ileal mucosa after exposure to theophylline in the absence of glucose (a) and in its presence (b). Note the dilated intercellular spaces in the latter.

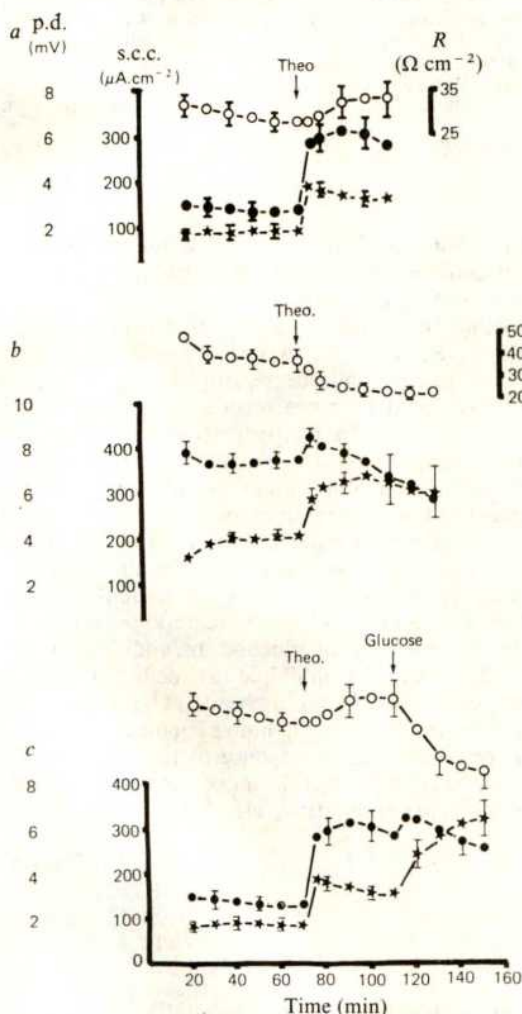
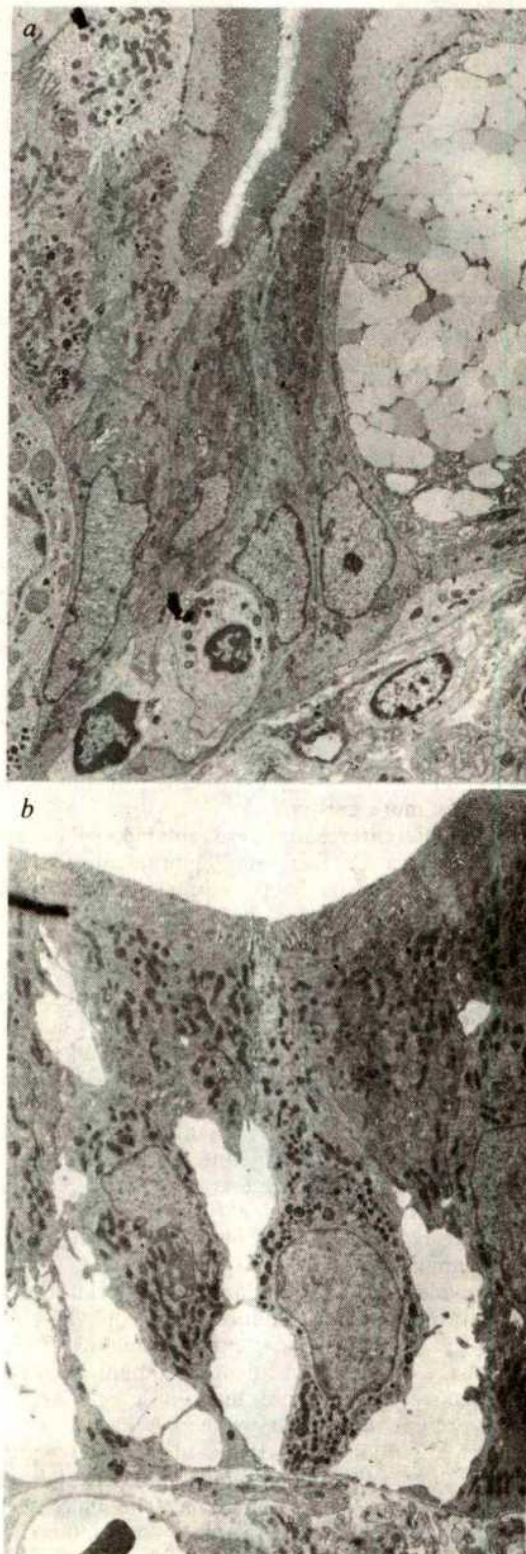


Fig. 1 Electrical responses of stripped human ileal mucosa to theophylline (10^{-2} M). Theophylline (Theo.) raises mucosal cyclic AMP concentrations by inhibiting its breakdown. Fresh mucosa, removed at operation for a variety of diseases, was separated from abnormal areas, stripped of muscle and serosa and mounted in Ussing type flux chambers as described previously⁸. In *a* glucose was absent from the bathing solutions; in *b* it was present throughout (15 mM), and in *c* it was added 40 min after the theophylline. In each case the p.d. (●) and s.c.c. (★) rose in response to theophylline but the calculated tissue resistance (*R*) (○) rose in the absence of glucose and fell in its presence. Glucose alone did not influence resistance but it fell when glucose was added after theophylline (*c*) indicating that this effect is dependent on the presence of both glucose and theophylline.

Table 1 Influence of theophylline (10^{-2} M) on ion transport ($\mu\text{mol cm}^{-2} \text{h}^{-1}$) in stripped ileal mucosa in glucose-free and glucose-containing media and across jejunal mucosa in glucose-containing media

	J Na ms	J Na sm	J Na net	J Cl ms	J Cl sm	J Cl net	J R net	I.s.c.
Ileum								
No glucose ($n = 11$) (control)	11.2 ± 1.0	9.52 ± 0.72	+ 1.68 ± 0.75	9.59 ± 0.98	10.93 ± 0.4	- 1.34* ± 0.71	+ 0.80* ± 0.45	3.82 ± 0.42
Theophylline	9.12 ± 0.70	10.99 ± 0.78	- 1.87 ± 0.89	8.00 ± 1.00	13.68 ± 0.69	- 5.68 ± 0.6	+ 1.94* ± 0.83	5.75 ± 0.69
<i>P</i>	0.05	< 0.005	< 0.01	< 0.05	< 0.001	< 0.001	NS	< 0.005
Glucose ($n = 9$) (control)	20.1 ± 1.9	8.3 ± 0.3	+ 11.8 ± 1.7	12.4 ± 0.9	8.8 ± 0.6	+ 3.6 ± 0.8	+ 05.* ± 1.2	8.7 ± 1.1
Theophylline	20.9 ± 2.6	11.3 ± 1.1	+ 9.6 ± 2.2	10.5 ± 1.3	13.3 ± 0.9	- 2.8 ± 1.2	+ 0.3* ± 2.2	12.7 ± 1.8
<i>P</i>	NS	< 0.02	NS	NS	< 0.01	< 0.01	NS	< 0.01
Jejunum								
Glucose ($n = 7$) (control)	14.5 ± 1.3	6.8 ± 0.7	+ 7.7 ± 1.6	7.7 ± 0.7	9.2 ± 0.5	- 1.5* ± 0.7	- 1.6* ± 0.7	7.6 ± 0.7
Theophylline	15.2 ± 1.7	8.6 ± 0.8	+ 6.6 ± 1.8	7.9 ± 0.9	12.0 ± 1.0	- 4.1 ± 0.7	+ 1.1* ± 1.1	11.8 ± 1.0
<i>P</i>	NS	< 0.01	NS	NS	< 0.01	< 0.01	< 0.02	< 0.001

1.5 $\mu\text{Ci } ^{22}\text{Na}$ and 2.5 $\mu\text{Ci } ^{36}\text{Cl}$ were added to the mucosal solution of one of a pair of tissues and the serosal solution of the other of the pair and, after 30 min equilibration, mucosal and serosal reservoirs were sampled at 20-min intervals. Tissues with electrical resistance differing by less than 25% only were paired to obtain net flux results and electrical values were averaged to yield a single value for each pair. Unidirectional and net fluxes were calculated as previously described⁸. Results are shown for theophylline-treated tissues and paired control tissues in which tissue resistance remained stable throughout the flux experiments. *n*, No. of tissue pairs; + indicates net absorption; -, net secretion. *R*, calculated tissue resistance; *J*ms, mucosal to serosal flux; *J*sm, serosal to mucosal flux; *J*net, net flux. Values are means $\pm$ s.e.m. NS, Not significant.

*Not significantly different from zero.

transport in the presence and absence of glucose (Table 1). In glucose-free buffer theophylline converted a net ileal absorption of sodium to a significant net secretion and chloride secretion was also induced. These changes were due to an increase in unidirectional flux from serosa to mucosa and a reduction in the opposite flux. The results are compatible with both an inhibition of a neutral sodium chloride influx and stimulation of a sodium chloride secretion. But, the decreased epithelial conductance could conceivably account for the reduced Na-Cl influx without invoking any other inhibitory mechanism. However, the increased flux towards the mucosa occurred despite the decreased conductance and the evidence for this secretory process is thus more certain.

Somewhat different results were obtained when glucose was present (Table 1). Here net chloride absorption was changed to secretion but net sodium transport was not significantly reduced. Thus it is possible that theophylline stimulated an uncoupled Cl secretion when glucose was present. The rise in s.c.c. induced by theophylline with glucose ($150 \mu\text{A cm}^{-2}$) was greater than without it ($100 \mu\text{A cm}^{-2}$) in these unpaired studies, possibly due to the separation of anion and cation transport and supporting the possibility of a rheogenic Cl secretion. Furthermore, Sheerin and Field¹⁵ observed that the pH of solutions bathing rabbit ileum influenced the sodium but not the chloride transport response to theophylline. The dependence of sodium secretion on pH suggests that it is not closely coupled with chloride secretion. An uncoupled chloride secretion in response to acetylcholine has also been demonstrated in human intestinal mucosa¹⁶.

But, the evidence for an uncoupled Cl secretion depends critically on the apparent failure of theophylline to influence Na transport in the presence of glucose. It remains possible that the marked movement of sodium from mucosa to serosa induced by glucose masked a relatively small reversal of sodium movement by theophylline.

The increased unidirectional flux of Na-Cl towards the mucosal side due to theophylline plus glucose, might reflect the increased conductance of mucosa. The increase in Cl flux was greater than that in Na (4.5 compared with $3.0 \mu\text{mol cm}^{-2} \text{h}^{-1}$). But, while this discrepancy could be

due to the additional stimulation of a mucosally-directed Cl pump, it could equally be due to a change in the relative conductance of Na and Cl (ref. 6).

Glucose-stimulated bulk fluid transport probably induces appreciable solvent drag forces which increase the mucosa to serosa flux of ions while decreasing that in the opposite direction. This would be more obvious when mucosal conductance is increased. In contrast to this prediction, the Na-Cl flux from mucosa to serosa remained unchanged, suggesting that theophylline might be inhibiting this flux.

In summary, these observations suggest that theophylline exerts several effects. First, it stimulates a sodium and chloride secretory process, seen best in the absence of glucose. This Na-Cl secretion may be a neutral coupled process but a rheogenic chloride secretion may also be revealed in the presence of glucose. In addition, a Na-Cl absorptive flux may be inhibited as deduced from the experiments performed in the presence of glucose.

Finally, it is of interest that human jejunal mucosa also actively secretes chloride in response to theophylline (Table 1) despite the fact that chloride movement here is entirely passive in control conditions, *in vivo*¹⁷ and *in vitro*¹⁸.

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Fast motor units are not preferentially activated in rapid voluntary contractions in man

MOST mammalian muscles include muscle fibres of different histochemical types which are organised in separate motor units (a motor unit is a group of muscle fibres innervated by a single motoneurone) with different physiological characteristics¹. When a muscle carries out a slowly graded contraction, the motor units of the slow type are activated before the motor units with a faster contraction time which are also the ones developing more force since they include a larger number of muscle fibres². This orderly recruitment can also be shown in intact man in whom the spike-triggered averaging method further allows the extraction of the mechanical twitch of a single motor unit from the whole muscle force³. In view of the versatility of the human hand movements and considering recent inferential views about variations in the recruitment order of motor units^{4,5}, we had anticipated that the fast motor units could be preferentially recruited in brisk voluntary contractions. This expectation is not borne out by the results presented. On the contrary we found the faster motor units to be recruited after the slow motor units, even in fast contractions.

A total of 54 single motor units were studied in the first interosseus muscle of the hand in five normal adult subjects. Highly selective bipolar electrodes were inserted through the skin and carefully positioned in the muscle belly so as to pick up selectively the action potentials of two to five different motor units at a single site. Each motor unit was clearly identified from background activities, even during strong rapid contractions. The hand was firmly held in a sturdy receptacle. The isometric force for abduction of the index finger was transduced with an Elf-1000-250 Flatline load cell. The mechanical twitch of each motor unit was extracted from the isometric myogram of the steadily contracted muscle by averaging the (FM tape recorded) analogue force signal. The action potential of the chosen motor unit was selected by a window trigger electronic circuit and made to initiate the duty cycle of a Nicolet model 1074 averaging digital computer. Several periods of steady contraction of the muscle were used in order to avoid fatigue effects. Due precautions were taken to automatically exclude mechanical interference from close-range firing of the unit⁶.

The isometric twitches with different contraction kinetics (time-to-peak 65 and 39 ms respectively) shown in Fig. 1; *a, b* correspond to two different motor units recorded with the electrode in the same position in the muscle. When the subject contracts his muscle progressively so that the force increases slowly as a ramp function reaching its peak in about 10 s, the slower contracting unit is activated first, and the faster unit is not recruited until several seconds later when the contraction force exceeds 280 g in this example. The sequence is highly reproducible for each recording site⁶.

In brisk contractions the timing of the motor units discharges is difficult to relate with precise levels of the fast-rising muscle force. But a consistent force threshold

of any chosen motor unit can be estimated by requesting the subject to produce a large series of very brief ballistic contractions, each achieving different peak forces from about 10 to 2,000 g. A given unit never discharges for brisk contractions below a certain peak force, while it always fires for contractions with peak force above that value. The method thus facilitates measurement of a true ballistic force threshold which is consistent in repeated trials⁷. The sample records of Fig. 1 *d-g* show that the smaller unit failed to discharge for the weakest contraction (*d*), but was activated in all others, while the larger unit only started firing when the ballistic peak force exceeded 80 g (*g*).

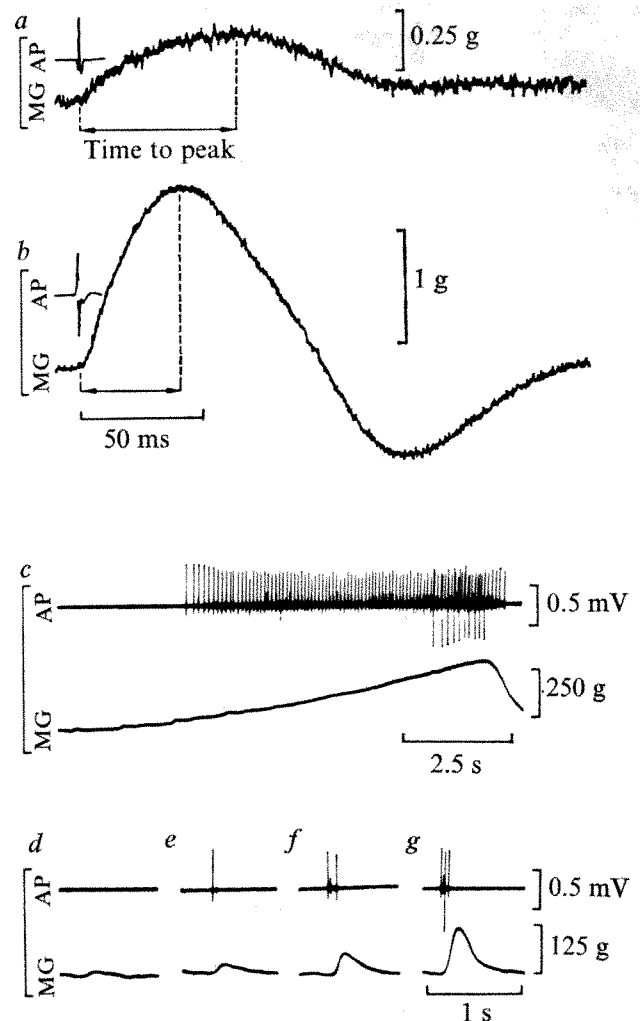
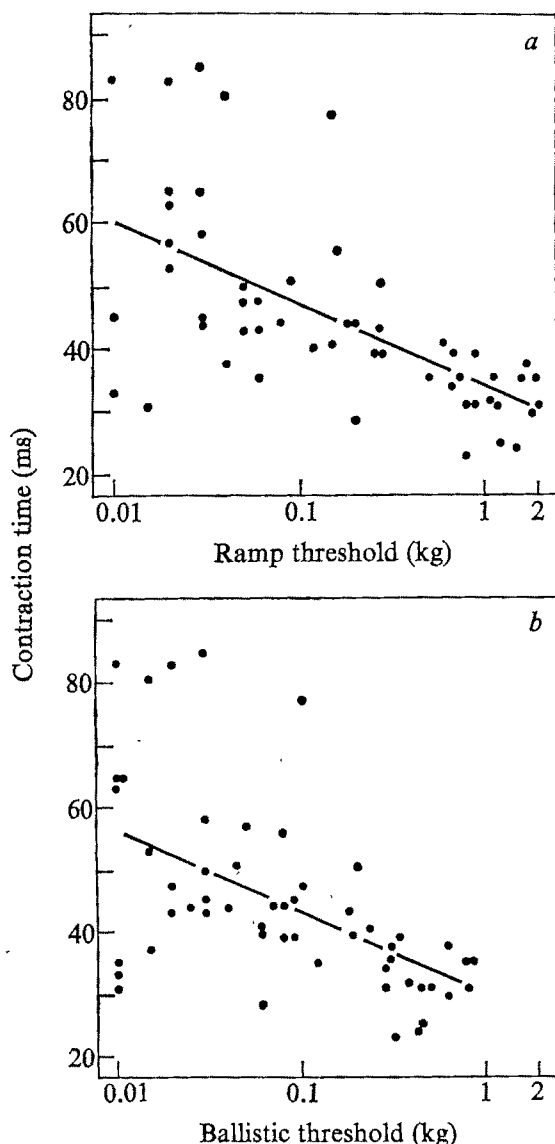


Fig. 1 Recruitment order and force threshold of two motor units in the first interosseus muscle. Normal subject, 28 yr old. The selective recording electrode allowed the action potentials of these two units to be clearly isolated from background activities. The isometric twitch of each unit was extracted by spike-triggered averaging of 512 discharge samples and presents a contraction time (time-to-peak) of 65 ms (*a*) and 39 ms (*b*) respectively. The peak forces are 0.3 g (*a*) and 1.5 g (*b*). The corresponding averaged action potentials (AP) are shown on the same time scale above the myogram (MG). *c*, Voluntary contraction of the interosseus muscle as the subject is tracking a slowly rising force ramp which achieves 300 g in about 10 s. The slower motor unit (illustrated in *a*) starts discharging when the ramp force reaches 20 g while the other motor unit which can easily be identified by its action potential waveform presenting a larger positive-going (downward) component (see *b*, 'AP') only starts discharging later, when the ramp contraction reaches 280 g. *d-g*, ballistic voluntary contractions recorded during the same experiment. The isometric peak forces are 10 g (*d*), 20 g (*e*), 50 g (*f*) and 110 g (*g*) respectively. The contraction in (*e*) just exceeds the ballistic force threshold of the slower motor unit studied. The ballistic contraction in (*g*) recruits the faster motor unit.

For each of the single motor units studied, we obtained complete data for the twitch contraction time, and for the force thresholds respectively in graded ramp and ballistic contractions. Confirming previous results⁴, we found that all the units with a higher ramp threshold were fast contracting. The units with a low ramp threshold were either fast or slow contracting but a significant relation was observed between the two features ($r_{xy} = -0.62$) (Fig. 2a). For the brisk contractions, we found the relation between ballistic force threshold and the twitch contraction time to be rather similar and also quite consistent ($r_{xy} = -0.54$) (Fig. 2b).

The pooled data of Fig. 2 do not allow us to exclude any possible change in recruitment order in the two types of voluntary contractions. This has been achieved by considering pairs of motor units recorded during the same trials from a given position of the recording electrode in

Fig. 2 Relation between the contraction time of the twitch of 54 different single motor units recorded in the first interosseus muscle (ordinate), and the force threshold of each unit (abscissa, logarithmic scale) estimated either with a slow tracking ramp contraction (a), or with brisk ballistic contractions (b). The calculated regression lines fit the functions: $y \text{ (ms)} = 34 - 13 \log x \text{ (kg)}$ (a) and $y \text{ (ms)} = 30 - 13.4 \log x \text{ (kg)}$ (b) respectively. The graph also shows that the absolute force thresholds are smaller in ballistic than in ramp contractions.



the muscle. Since the specific question considered was whether any reversal of recruitment occurred in brisk contractions, the motor unit pairs considered were required to present significantly different twitch kinetics which we arbitrarily chose to define as a difference of at least 8 ms (range 8–56 ms; mean 23 ms) between their respective contraction times. Twenty-two pairs were retained which satisfied the further criterion that, for any pair, the slower unit had been recruited before the faster unit of the pair in the slow ramp contractions. This was obviously required to answer the question whether any preferential activation of the faster unit occurred in ballistic contraction. However, we did not observe a single instance in which the faster unit of the pair was recruited before the slower unit when the subject produced a brisk ballistic movement instead of a slow tracking ramp.

The wide repertoire of voluntary movements in man and the capacity for brisk and accurate finger displacements is related, amongst other things, to the richness of corticomotoneuronal (pyramidal) connections^{9,10}. When man contracts a muscle so as to track a slowly rising ramp force, the recruitment order of the single motor units is consistent and stereotyped^{6,8} (Fig. 2a). Such movements, even though they are carried out through pyramidal commands on the segmental α motoneurons, involve a co-activation of the γ motoneurons which contract the spindle muscle fibres and thereby elicit an important excitatory drive through the γ loop on to the same α motoneurons¹¹. Spindle afferents feedback excitation was shown in animals to recruit small α motoneurons before larger ones, according to Henneman's size principle^{3,12}. It is thus not surprising that ramp voluntary contractions in man should also obey the size principle. By contrast, when man executes brisk contractions as fast as possible, the pyramidal commands to the α motoneurons must be pre-programmed so as to produce the required ballistic force, and there is no time for any corrective feedback action from spindle or other afferents¹². In such conditions, the synaptic input to these α motoneurons is different from that in ramp contractions, and we expected to find more flexibility in the recruitment order, if only for allowing fast contracting motor units to be preferentially activated in brisk movements, but our present data definitively exclude such an hypothesis. Both slow and fast motor units containing fibres of either histochemical types respectively occur in the human interosseus muscle¹⁴. The muscle fibre typing is known to be related to the motoneurone properties, including cell size¹. Our findings suggest that the significance of the histochemical and functional types of motor units (slow and fast) should be related, not to any selective usage in either slow or fast voluntary movements in man, but rather to the more frequent activation of the smaller motoneurons according to the size principle¹⁵.

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TEA binding sites in crayfish axon membranes

VOLTAGE-DEPENDENT ion channels are the basis of nerve impulse propagation along the axon. The mechanism of gating and ionic selectivity of these channels remains obscure as long as the membrane molecules participating in these processes are unknown. Some biochemical properties of a tetrodotoxin binding component of the sodium channel have been determined¹, but for the potassium channel not even the number of channels per unit axon surface is known. From noise measurements it has been estimated that the squid giant axon contains about 60 K⁺ channels μm^{-2} (ref. 2), but this figure remains to be confirmed by other methods. Here I report experiments showing that specific and covalent labelling of tetraethylammonium (TEA) binding sites with a radioactive photoaffinity label can be achieved. 4-Azido-2-nitrobenzyltriethylammonium fluoroborate has been shown to block selectively, and after irradiation irreversibly, potassium conductance in the node of Ranvier of frog sciatic nerve³. I present here evidence that in crayfish unmyelinated nerve fibres, the label used reacts with a hydrophobic area at the mouth of the potassium channel postulated by Armstrong⁴.

Walking nerve from *Astacus leptodactylus* was cut into small pieces and 50 mg of tissue was homogenised in 0.2 ml Ringer's solution with a glass Micro-Homogeniser (Micro-Metric Instrument Company, USA). The homogenate was washed three times by centrifugation and resuspension to remove the soluble proteins. The washed membranes were incubated with the photoaffinity label (³H activity 100 mCi mmol⁻¹, synthesised by H. Kiefer, Basle) at final concentration as indicated in the legends to the Figures. After 10 min pre-incubation in the dark, irradiation was performed with an ultraviolet lamp (Sterisol, Quarzlampen GmbH, Hanau, Germany) mounted 5 cm above the sample. The irradiated sample was washed three times by centrifugation and resuspension to remove non-covalently bound photolabel. The labelled membranes were extracted overnight with 1% Triton X-100 in Ringer's solution containing 0.1 mM phenylmethylsulphonylfluoride and 0.02% sodium azide. After 30 min centrifugation at 40,000g the Triton extract was submitted to SDS-polyacrylamide gel electrophoresis⁵.

Figure 1a shows that apart from radioactive material not entering the 10% polyacrylamide gel, there is only one radioactive component migrating slightly slower than the tracking dye. None of the protein bands in the gel contains significant amounts of label. The residual membrane proteins which could not be extracted with Triton (Fig. 1b) were not labelled either. Figure 1c shows that analysis of a labelled membrane which does not contain voltage-dependent potassium channels, namely the postsynaptic membrane from *Torpedo californica* electric tissue. Here the radioactive band of Fig. 1a is missing; instead all the protein bands which have been shown to be part of the acetylcholine receptor complex are labelled. (This is in agreement with the affinity of the acetylcholine receptor for photolabels containing quaternary ammonium groups⁶) The three experiments of Fig. 1 indicate that the photolabel reacted selectively with a component typical for the excitable membrane of the crayfish nerve, presumably a

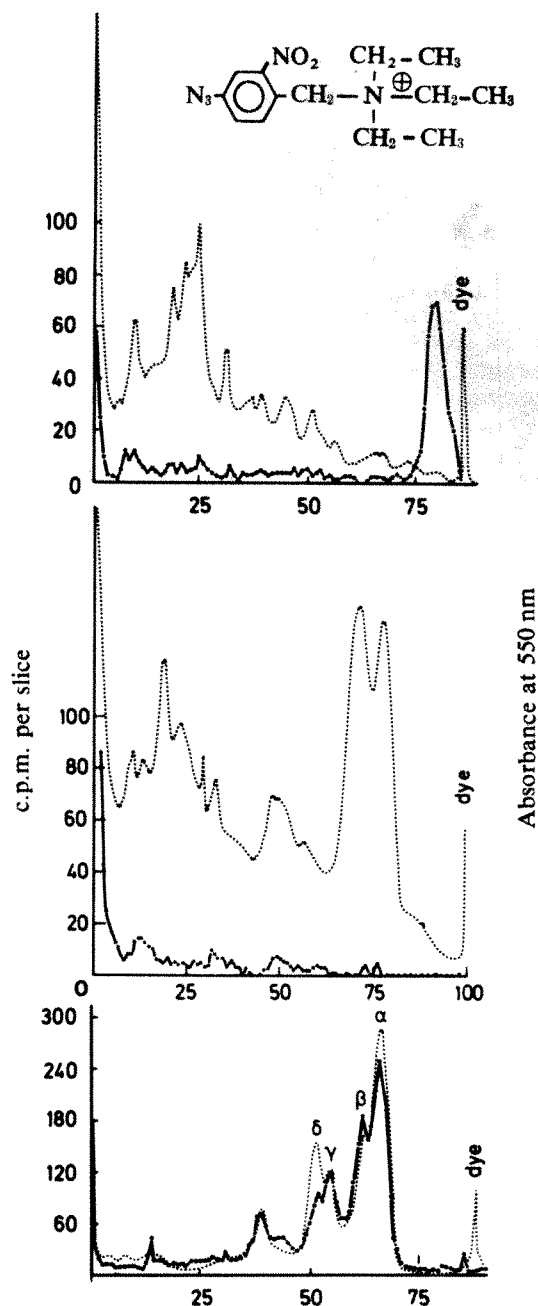


Fig. 1 SDS-polyacrylamide gel electrophoresis of crayfish nerve membranes labelled with the photoaffinity label 4-azido-2-nitrobenzyltriethylammonium fluoroborate. *a*, Absorbance (---) and radioactivity per mm gel slice of a 10% polyacrylamide gel. Walking nerve membranes of *Astacus leptodactylus* were labelled by 2-min irradiation at room temperature of an incubation mixture containing about 1 mg ml⁻¹ membrane protein and 0.5 mM tritiated photolabel in Ringer's solution. Electrophoresis (from left to right) was performed with a Triton X-100 extract of this membrane preparation containing 5 μg protein, at 8 mA per gel. The gels were stained with Coomassie blue. The main radioactive component migrated slightly slower than the tracking dye, the bulk of the lipids (visible as a white band) slightly faster. *b*, Absorbance and radioactivity of a gel of the membrane after Triton extraction. After centrifugation of the axon suspension in 1% Triton X-100 the Triton-insoluble pellet was dissolved in 1% SDS containing 10⁻³ M dithiothreitol. Electrophoresis as in *a*, with 5 μg protein on the gel. *c*, Control with a membrane not containing voltage-dependent potassium channels. Postsynaptic membrane fractions rich in acetylcholine receptor prepared from *Torpedo californica* electric tissue⁷ at a concentration of 0.2 mg ml⁻¹ in Ringer's solution were incubated with 0.5 mM photolabel and irradiated for 2 min. Electrophoresis with 10 μg of the labelled protein was the same as in *a* and *b*. Note that the main radioactive band of the upper gel is missing. Instead, the main proteins of the receptor membrane α - δ reacted with the label although not equally: the δ component incorporated only little radioactivity (as observed before⁸) again confirming the selectivity of the photoaffinity label.

tetraethylammonium (TEA) binding component of the potassium channel.

As further proof of this assumption two criteria of the specificity of a label for a given binding site were investigated: interaction with specific drugs and saturability. Figure 2 shows the concentration dependence of the ligand binding. Saturation was accomplished at an extrapolated incorporation of 12.5 pmol photolabel per μg protein extractable by Triton X-100 and from this result an attempt was made to estimate the density of binding sites in the axon membrane. From electron microscope pictures of a cross section of the nerve (performed by Ms Cahill in Professor Florey's laboratory) we estimated the axon surface area of 1 cm of the nerve. This amount of nerve incorporated 312 pmol of the label or 59×10^{-11} pmol μm^{-2} ; which represents 354 binding sites μm^{-2} . Size and number of fibres varies by orders of magnitude over the cross

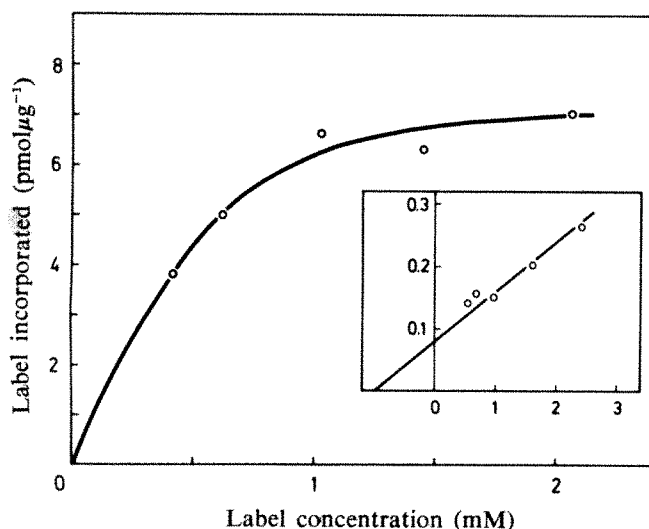


Fig. 2 Concentration dependence of the incorporation of photolabel into crayfish nerve membranes. The ordinate refers to the main radioactive band in the SDS-polyacrylamide gel of membranes prepared, labelled and extracted with Triton X-100 as described. The inset shows the reciprocal plot of the data of the main figure. Extrapolation suggests saturation at 12.5 pmol label per μg protein in the Triton extract. Standardisation with the amount of extractable protein is not intended to suggest that the labelled molecule is a protein. The radioactive band in the gel in fact probably represents a lipid associated with proteins *in situ*.

section of the nerve and so the results from several fairly representative areas were averaged for the calculation. Taking parts of the nerve with small fibres, that is, with large axon surface area, the number of binding sites is about $220 \mu\text{m}^{-2}$. This still seems to be high but since it is preliminary, analogous experiments with squid giant axons are under way.

TEA-C9, the strong inactivator of potassium conductance investigated by Armstrong⁴, protects the membrane against incorporation of photolabel (Table 1). A larger protective effect cannot be expected because during 1 min irradiation the reversible ligand TEA-C9 competes with the covalently reacting photolabel. Treatment of the Triton X-100 extract before the SDS electrophoresis with phospholipase A₂ from hog pancreas (Boehringer, Mannheim, Germany) reduces by about 75% the recovery of radioactivity from the gel; phospholipase D, trypsin and chymotrypsin have no effect. The total radioactivity can be extracted from the membrane with chloroform-methanol 2:1. These results seem to indi-

Table 1 Effect of the TEA analogon TEA-C9 in which one of the ethyl groups is substituted by a nonyl residue

Photoaffinity label (mM)	TEA-C9 (mM)	Inhibition (%)
0.5	10	19
0.25	10	67

The reagent was supplied by E. Rojas, Norwich, England.

cate that the labelled molecule is a phospholipid or a small proteolipid. Armstrong has shown that substitution of one ethyl group of TEA by more hydrophobic residues increases its potency as inactivator of the potassium conductance. This is in agreement with a model of the channel that postulates a hydrophobic area at its mouth. The results presented here may indicate that the photoaffinity label reacted with the lipid rim of the funnel-like entry⁴ to the potassium channel.

The claim that the photolabel selectively reacted with components of the potassium channel is based on three observations: The saturation curve obtained with increasing label concentrations; the competition with the specific potassium conductance inactivator TEA-C9; and the electrophysiological experiments with the frog sciatic nerve³. This last is based on the assumption that the pharmacological properties with respect to TEA and its derivatives are similar in the crayfish axons used here and the frog sciatic nerve investigated previously³. These experiments suggest that photoaffinity labelling might be a valuable tool for the determination of ion channel densities and channel structure in various tissues.

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Pentylentetrazol and penicillin are selective antagonists of GABA-mediated post-synaptic inhibition in cultured mammalian neurones

PENTYLENETETRAZOL (PTZ) and penicillin (PCN) are potent convulsants commonly used to produce focal or generalised epileptiform discharges in the mammalian central nervous system¹. Investigations of the cellular basis for their convulsant effects have been performed in various vertebrate and invertebrate preparations with two basic hypotheses dominating the literature: (1) that the convulsant effect is

produced by pharmacological actions on synaptic transmissions to reduce inhibition²⁻⁸ and/or to enhance excitation⁹, or (2) that the convulsants directly enhance the excitability of neuronal membranes apart from effects on synaptic transmission¹⁰⁻¹⁶. We have studied the mechanism of their convulsant action in a mammalian tissue culture system and report here that both PTZ and PCN selectively antagonise post-synaptic responses to γ -aminobutyric acid (GABA), a putative inhibitory neurotransmitter¹⁷. The results presented here provide direct evidence derived from the mammalian nervous system that the convulsant action of PTZ and PCN may be due to a specific pharmacological action on GABA-mediated inhibitory synaptic transmission.

Mouse spinal cords were dissected from 12-14-d-old mouse embryos and mechanically dissociated; the cells were plated at a density of about 10^6 cells per 35-mm diameter dish and grown in tissue culture for at least 4 weeks before electrophysiological study¹⁸. Mature cultures at this age contain numerous well-differentiated neuronal cell types but in these experiments only the larger diameter (10-50 μ m) multipolar spinal cord (SC) nerve cells were studied. SC cells were penetrated under direct vision on a modified stage of an inverted phase contrast microscope with either 3 M KCl or 4 M K acetate microelectrodes (25-50 M Ω). Use of a conventional bridge circuit allowed simultaneous recording of membrane potential and injected current. All recordings were made in the normal growth medium (90% modified Eagle's medium/10% horse serum); MgCl₂ was added to a final concentration of 10 mM to suppress spontaneous synaptic activity and thus allowed a clearer examination of postsynaptic pharmacology. Glutamate (0.5 M pH 8), GABA (0.5 M pH 3.5), glycine (0.5 M pH 3.5), β -alanine (0.5 M pH 3.5), Na penicillin (0.3 M pH 5.6) (all Sigma) and PTZ (0.3 M pH 6) (K & K) were iontophoresed from high resistance (50-100 M Ω) theta glass micropipettes.

Both PTZ and PCN rapidly and reversibly antagonised the voltage response and conductance change evoked by iontophoresis of GABA without affecting responses to either glycine, β -alanine or glutamate (Fig. 1). The depression of the GABA response by concurrent iontophoresis of convulsant was observed on 25 SC cells with PCN and on

20 SC cells with PTZ, and in both cases the depression was dose dependent. The selectivity of either convulsant's antagonism of GABA responses has been observed on 20 cells examined with both GABA and either glycine, β -alanine or glutamate. Furthermore, PCN applied iontophoretically at currents which produced inhibition of the GABA response did not significantly alter resting membrane potential, input resistance, the inversion potential of the GABA response or the characteristics of the action potential (unpublished observations). Similar observations were made with PTZ except for a slight increase in input resistance noted at high currents. Thus, iontophoretically applied, both PCN and PTZ selectively depress GABA-mediated post synaptic responses on mammalian spinal neurons at doses which have no effect on other membrane properties.

Analysis of GABA dose-response curves obtained during control conditions and during coincident iontophoresis of PTZ or PCN suggests that the convulsants inhibit the GABA response competitively. In addition, the manifest selectivity of both convulsants for GABA also suggests that they both compete for GABA at the GABA receptor and not at the chloride conductance mechanism, as has been suggested previously⁷ for PCN. The finding that PTZ selectively blocks the GABA response in mammalian cultured neurones is in contrast to observations made in the frog spinal cord⁸ where PTZ antagonises the dorsal root potential and the action of GABA, β -alanine and taurine on primary afferent fibres with no ability to antagonise the hyperpolarising responses of these neutral amino acids on motoneurons. The results thus indicate that PTZ and PCN are GABA-specific antagonists in the mammalian central nervous system and support the hypothesis that part of their convulsant action is based on a selective blockade of GABA-mediated inhibition.

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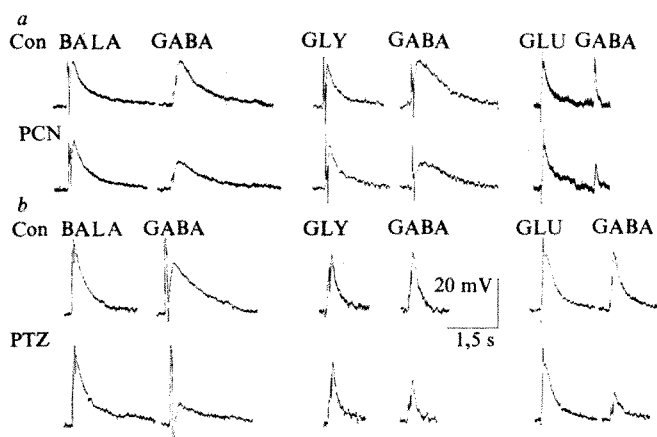


Fig. 1 PCN (A) and PTZ (B) selectively antagonise GABA-mediated postsynaptic responses in cultured mammalian neurones without affecting responses to β -alanine (BALA), glycine (GLY) or glutamate (GLU). All recordings were made using 3 M KCl micropipettes with the cell hyperpolarised to about -90 mV. In these conditions, the inversion potentials for all amino acid responses are more depolarised than -30 mV, and thus all responses shown are depolarising. The amino acids were iontophoresed with 50-ms pulses using micropipettes positioned close to PCN and PTZ pipettes at the neuronal surface. In each case shown, the GABA response was depressed by PCN or PTZ with no change occurring in the other amino acid responses. The 1-s time bar is applicable to all responses except for the GLU and GABA responses in a. Con, control traces.

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Reactivation of murine cytomegalovirus by cyclophosphamide

THE high prevalence of cytomegalovirus (CMV) infection in transplant recipients is well known but our understanding of the risk factors involved is incomplete. It is thought that the role of immunosuppression is probably paramount. In a series of rheumatologic patients followed prospectively

Table 1 Reactivation of MCMV in DBA/2 mice by cyclophosphamide

	No. of cyclophosphamide injections			
	0	1	2	3
Salivary gland	0/10*	0/4	11/13	8/8
Kidney	0/10	0/4	3/13	0/8
Lung	0/10	0/4	2/13	0/8
Spleen	0/10	0/4	0/13	0/8
Liver	0/10	0/4	0/13	0/8

MCMV was assayed by plaque formation on monolayers of mouse embryo cell cultures under an overlay containing 0.8% gum tragacanth⁴. Virus titres in the salivary gland ranged from 2.18 to 3.54 log₁₀ PFU per 10 mg of tissue.

*No. of animals with virus in organ/total.

after initiation of cyclophosphamide therapy, a significant proportion (43%) developed CMV infection as evidenced by viruria and serologic response¹. Since most of these infections were seen in initially seropositive patients, we believe that they represent reactivations of latent (herein defined as an infection in which no virus can be detected by standard plaque assays) or inapparent infection. Presumably, reactivation by immunosuppression also occurs in transplant recipients. Another risk factor for CMV infection in renal transplant recipients is the transfer of kidneys from donors seropositive for CMV into seronegative recipients^{2,3}. We are using the mouse cytomegalovirus (MCMV) model for better understanding of the factors involved in latency and reactivation of this group of herpes-like viruses and have shown that allografting will enhance chronic MCMV infection in the recipients⁴. We present here further observations on the mouse model, showing that latent MCMV infection can be reactivated by treatment with cyclophosphamide and that the transfer of latently infected spleen cells can lead to infections in recipients. We also show a role for immunosuppression in the latter situation.

Latent infections were developed by injection of 5–6-week-old DBA/2 inbred male mice (Jackson Labs) with 2×10^5 plaque-forming units (PFU) of MCMV, Smith Strain, intraperitoneally (i.p.). Free virus was no longer detectable in the spleen by 6 weeks after infection. Lungs, liver, kidney and salivary glands were clear by 16 weeks.

Animals that had been infected 20–24 weeks previously were treated with one, two and three doses of cyclophosphamide (150 mg per kg body weight) 5–6 d apart. Their organs were removed, homogenised as 10% suspensions in cell culture medium, clarified by centrifugation and titrated 2 weeks after the last dose (Table 1). Untreated control mice and mice which received only one injection of cyclophosphamide had no detectable free virus in any of the organs tested. On the other hand, 11 out of 13 mice receiving two doses of the drug and all mice treated after three doses reactivated CMV in the salivary gland. The total number of mice in which MCMV was reactivated is

the same as the number of salivary glands positive (Table 1), since MCMV was not found in other organs of mice with MCMV-negative salivary glands. The finding of virus almost exclusively in the salivary gland does not necessarily mean that it is the site of reactivation, as virus in any part of the animal might migrate to the salivary gland, the favoured site of virus replication.

Although investigators could not reactivate herpes simplex virus infections in sensory ganglia of mice using cyclophosphamide⁵, the results presented in Table 1 indicate that cyclophosphamide treatment can reactivate inapparent or latent CMV infections. There is, however, a small but important possibility that the results were produced by amplification of a smoldering but undetected lytic infection. Indeed this possibility is virtually impossible to eliminate.

In an attempt to establish the existence of a transferable, latent CMV infection, spleen cells from DBA/2 mice infected 8–12 weeks previously were used as donors in spleen cell transfer experiments. Recipient DBA/2 and C57BL/10 mice aged 6–10 weeks were treated with a single dose of cyclophosphamide (150 mg per kg) 48 h before injection with 10⁶ whole or sonically disrupted spleen cells. Four weeks later, salivary glands from normal and immunosuppressed mice were removed and titrated (Table 2). Transfer of latently infected DBA/2 spleen cells into syngeneic hosts resulted in frequent reactivation in similar proportions of both normal and immunosuppressed animals. Transfer of the same cells into allogeneic C57BL/10 mice, however, resulted in infection in a significantly greater number of treated than untreated recipients ($P < 0.05$)⁶. Free virus was not detected by titration of disrupted donor spleen cells or by injection of 10⁶ sonically disrupted cells into immunosuppressed DBA/2 mice. As a control, we performed 'reconstruction' experiments by adding free virus to suspensions of spleen cells before disruption. Recovery of the virus was >80% using cells derived from either normal or latently-infected animals, suggesting that lytic virus was not inactivated by cell debris or sonication.

These results demonstrate that MCMV may produce latent infections in spleen cells and that this latent infection can be reactivated in other animals by cell transfer. Additionally, in allogeneic recipients, infections due to reactivation of the donated spleen cells were more frequent in cyclophosphamide treated mice than in untreated animals.

The reactivation of latent MCMV by cyclophosphamide provides supportive experimental evidence of the postulated role of immunosuppression in CMV infection of transplant recipients and other immunosuppressed patients^{1,2,7}.

The apparent reactivation of latently-infected spleen cells in non-immune recipients closely resembles the situation seen in kidney transplant recipients. The enhancing effect of cyclophosphamide in allogeneic recipients was probably due to the suppression of rejection of latently-infected transplanted cells, and survival of such cells seems to be the most important factor in determining the frequency of reactivation in this system. A direct effect of cyclo-

Table 2 Effect of transfer of latently-infected DBA/2 spleen cells to normal and immunosuppressed syngeneic and allogeneic recipients

Strain	Recipient Cyclophosphamide†	Inoculum†	Experiment no.				Total
			1	2	3	4	
C57BL/10	—	Live	0/5*	0/5	2/10	—	2/20
C57BL/10	+	Live	3/5	3/10	4/5	—	10/20
DBA/2	—	Live	2/5	2/5	3/5	7/15	14/30
DBA/2	+	Live	4/5	6/10	4/5	5/10	19/30
DBA/2	+	Sonicated	—	—	0/5	0/5	0/10

*No. of animals with virus in salivary gland/total. See Table 1.

†Live, 10⁶ spleen cells from latently-infected DBA/2 mice; sonicated, the same number of sonicated cells.

‡+, Cyclophosphamide-treated; —, untreated.

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The D2BAL-10 MuLV, isolated from a dimethyl(α)-benzanthracene-induced, transplantable DBA/2 leukaemia, was injected into newborn BALB/c mice. When they were 36 d old p30 was determined by radioimmunoassay* in the blood of individual mice (Fig. 1). In eight of 13 mice injected with virus we found significantly increased levels of blood p30, ranging from 760 to 1,200 ng ml⁻¹, whereas

One of the control animals with a low p30 concentration in the blood died after 192 d with enlarged lymph nodes and splenomegaly, possibly due to leukaemia. The remaining 12 control animals were alive without signs of disease

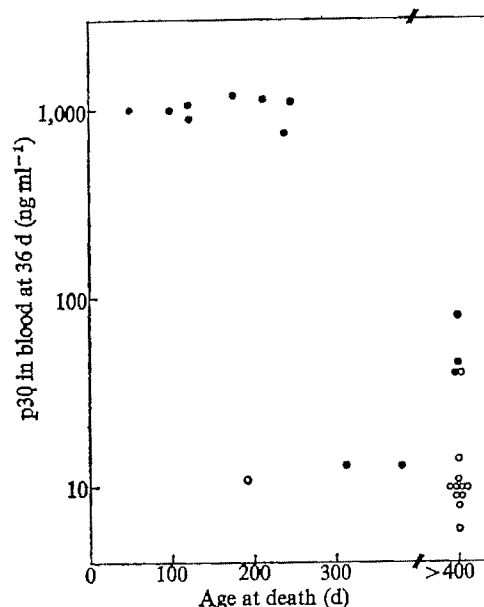


Fig. 1 Newborn BALB/c mice were injected with D2BAL-10 MuLV. Concentration of p30 at 36 d in blood from mice injected with virus (●), and from control mice receiving only buffer + DEAE-dextran (○) is shown in relation to later mortality. A cell-free extract, prepared from D2BAL-10 ascites cells (13 passages), was added to monolayer cultures of SC-1⁷ murine cells. Forty-eight-h collections of MuLV were concentrated 400-fold by producing a pellet of virus from the supernatant medium and resuspending in TEN buffer (20 mM Tris-HCl, 1 mM EDTA, 100 mM NaCl, pH 7.6) with p30 at about 0.4 mg ml⁻¹. DEAE-dextran⁶ was added to a concentration of 25 µg ml⁻¹. Each of 13 newborn (less than 24 h old) BALB/cABoM-Fib mice received 50 µl of the virus suspension subcutaneously on the back, and 13 newborn BALB/c received 50 µl of TEN buffer with DEAE-dextran (25 µg ml⁻¹). At 36 d a 25-µl sample of blood from each mouse was collected in one tenth volume of 100 mM NaEDTA, pH 6.5. The proteins were solubilised by addition of Triton X-100 to a final concentration of 0.4% followed by freezing and thawing. The radioimmunoassay mixture⁸ contained 0.5 ng of ¹²⁵I-Rauscher-MuLV p30, 12 nl of rabbit anti-FeLV, 1 µl of nonimmune rabbit serum and 10 µl of blood, or a standard dilution of Rauscher-MuLV p30, in a total of 120 µl of TENG/BSA (TEN buffer containing 0.2% Triton X-100, 0.1% NaN₃ and bovine serum albumin (5 mg ml⁻¹)). The antibody-bound radioactivity was precipitated by pig immunoglobulin against rabbit IgG (Dakopatts, Denmark), collected on membrane filters, washed and counted.

after 13 months. They had normal concentrations of blood p30 ($<50 \text{ ng ml}^{-1}$) after 200 d.

These results suggest that the D2BAL-10 MuLV isolated from a long-transplanted, chemically-induced DBA/2 leukaemia is oncogenic in BALB/c mice. It is also obvious that infection of newborn mice with the virus leads to high concentrations of the core protein, p30, in the blood within the first 5 weeks of life in most cases. The increased concentrations of p30 in the blood probably represent replication of the inoculated MuLV in the animals. Finally, there seems to be a significant correlation between early high p30 concentrations in the blood of otherwise healthy animals and the subsequent development of leukaemia. The results agree with the observation that quantification of p30 in the blood identifies among inbred strains of mice, a group which is at high risk of developing leukaemia⁶.

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Histone content of germinating pea embryo chromatin decreases as DNA replicates

MODIFICATIONS in the expression of genetic information in eukaryotes are probably controlled by changes in the proteins associated with chromatin¹. Unfortunately, in most of the differentiating systems which have been studied these changes are barely detectable, so that we do not know either which changes occur or when they occur. As a part of a more general study of the molecular developmental biology of seed germination we have investigated changes in chromatin of germinating pea embryo axes. Some particularly interesting features of this biological system make it useful for studying developmental changes in chromatin structure. First, it is quite easy to obtain large numbers of homogeneous seeds. Second, germination constitutes a switch from a dormant, arrested state to a new developmental programme. It is thus possible to study the sequence of molecular events which underlie the transition from a completely inactive genome to an active one. Third, this reactivation of the genome is accompanied by two waves of synchronous mitoses which can be considered as temporal markers. Finally, the germinating embryo constitutes a set of differentiating tissues. To our knowledge, no animal system shares all these features together. The results which are described in the following sections demonstrate that a considerable loss of histone per DNA unit is associated with this reactivation of the

genome during germination and that most of these changes take place as DNA replicates.

Histones from pea embryo chromatin were prepared as described previously; these had been characterised using polyacrylamide gel electrophoresis, electrofocusing and amino acid composition^{2,3}. Briefly, chromatin was prepared from a nuclei-enriched pellet. Most of the contaminating material was removed by several washings and the chromatin was further purified by dissociation in 2 M NaCl followed by gentle reassociation in 0.15 M NaCl. To prevent protease activity, sodium thiosulphate was included in all buffers, and all operations were carried out at 4 °C. That no degradation occurs is indicated by the SDS-polyacrylamide gel electrophoretogram shown in Fig. 1. Molecular weights of the individual histones were in accordance with previously published values^{1,4}.

We first determined the total histone/DNA ratio in

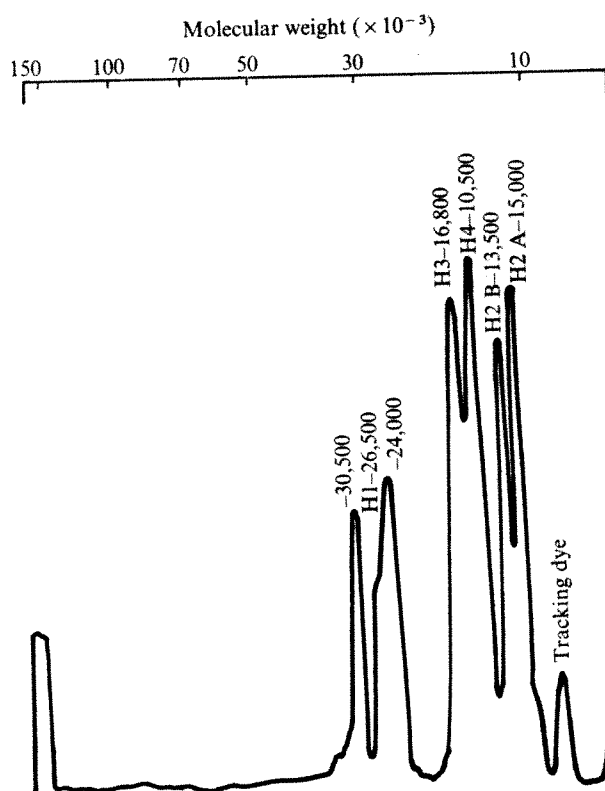


Fig. 1 SDS-polyacrylamide gel electrophoresis pattern of dry pea embryo histones. Chromatin was extracted by homogenisation of embryos in 0.05 M Tris buffer pH 8.0, containing 0.25 M sucrose, 0.001 M MgCl_2 , 0.01 M β -mercaptoethanol and 0.05 M sodium thiosulphate. After filtration of the homogenate through Miracloth, a nuclei-enriched pellet was obtained by centrifugation at 8,000g for 20 min. The pellet was washed three times, twice with the above buffer lacking sucrose and magnesium and finally with 0.01 M Tris buffer, pH 8.0, containing 0.002 M β -mercaptoethanol and 0.05 M sodium thiosulphate. The crude chromatin pellet was then dissociated in 0.01 M Tris buffer, pH 8.0, containing 2 M NaCl and 0.05 M sodium thiosulphate, and gently reassociated by dialysis against the same buffer lacking NaCl, to a final concentration of 0.15 M NaCl. Total histones were extracted by stirring twice with 0.25 N HCl for 2 h and precipitated with eight volumes of acetone at -20 °C for 24 h. After centrifugation, the histone pellet was washed successively with acetone and ether and air dried. Histones were analysed by electrophoresis on 12.5% acrylamide gels containing SDS prepared by the method of Laemmli and Favre⁵. Electrophoresis was at 3 mA per gel for 6 h, with bromophenol blue as tracking dye. Gels were stained with Coomassie Brilliant Blue R and scanned at 620 nm with a Chromoscan (Joyce Loebel) apparatus. Molecular weights were estimated by the procedure of Weber and Osborne⁶.

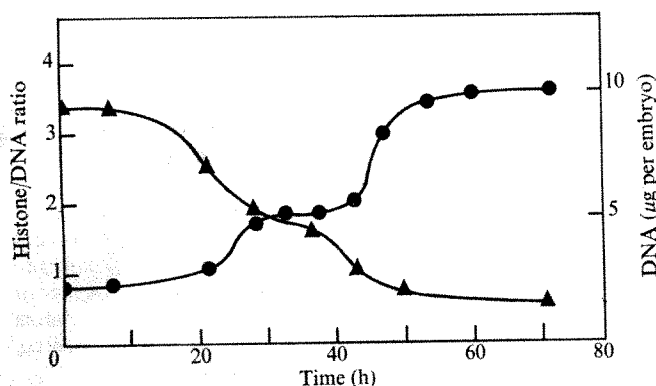
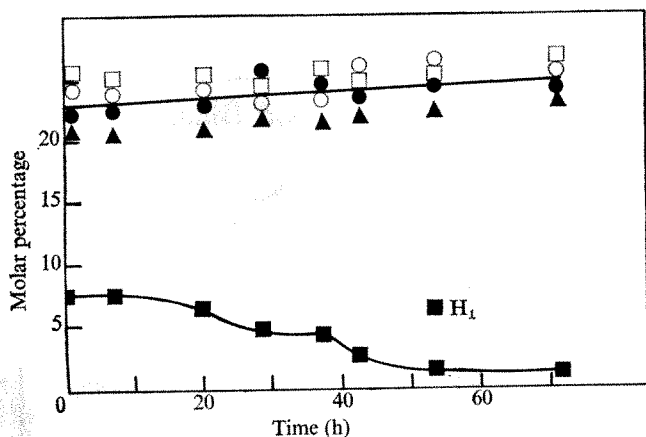


Fig. 2 Changes in DNA amount and in histone/DNA ratio during germination. DNA was extracted by the phenol-chloroform method of Marmur⁷, from embryos germinated for the required period, and assayed spectrophotometrically (●). Chromatin, prepared as described in Fig. 1, was divided into two parts: one for the DNA estimation by the above method, the other for histone extraction (as described in Fig. 1) and estimation by the Lowry method⁹. The histone/DNA ratio of chromatin was calculated (▲).

purified chromatin and examined its variation as a function of germination time. Figure 2 shows the changes in embryo DNA content and in the total histone/DNA ratio. DNA content increases in two steps corresponding to the two waves of mitosis. This has been confirmed by ¹⁴C-thymidine incorporation and cytological observation of numerous mitoses. The histone/DNA ratio curve demonstrates that the histone content of purified chromatin decreases as the germination proceeds. Strikingly, decreases in the ratio are synchronous with DNA synthesis, providing clear evidence that major changes in chromatin take place at the time of DNA replication. Note also that the large excess histone in the dry embryo is most likely accounted for by actual DNA-bound histones than by a storage form, since no free histone can be detected either in the different washings of

Fig. 3 Molar percentage of each histone as a function of germination time. Histones were isolated separately by the differential extraction procedure (method 2) of Johns⁸ and assayed by the method of Lowry⁹. In our hands, this yields a fraction corresponding to H2B partially contaminated by H2A. The percentage of contamination was evaluated by electrophoresis and the values corrected. Using the molecular weights as determined in Fig. 1, the molar percentage of each histone was calculated. O, H2A; □, H2B; ▲, H3; ●, H4.



the crude chromatin or in the cytoplasm, and since chromatin purified through a sucrose gradient yields the same high histone/DNA ratio (our unpublished results).

We then asked whether each individual histone behaves in the same way or not. Since an accurate quantitation of each individual histone is difficult to obtain by gel electrophoresis, due to differential dye binding, we fractionated the total histone preparation by a selective extraction procedure^{3,8}. Each fraction was checked for purity by gel electrophoresis and assayed by the Lowry method⁹. Using the molecular weights, we calculated the molar percentage of each individual histone and plotted them against time of germination. The data presented in Fig. 3 clearly demonstrate that four of the histones (H2A, H2B, H3 and H4) remain in equimolar proportions although their total amount per DNA unit decreased. This suggests that they remain associated together in a nucleosome-like structure at every stage of germination, including the waves of mitosis. On the other hand, H1 behaved independently. As the germination proceeded, the relative proportion of H1 decreased in steps which, again, showed a remarkable coincidence with DNA doublings.

A direct implication of these observations is that accessibility of DNA in chromatin to endonucleases should increase during the period considered. Chromatin corre-

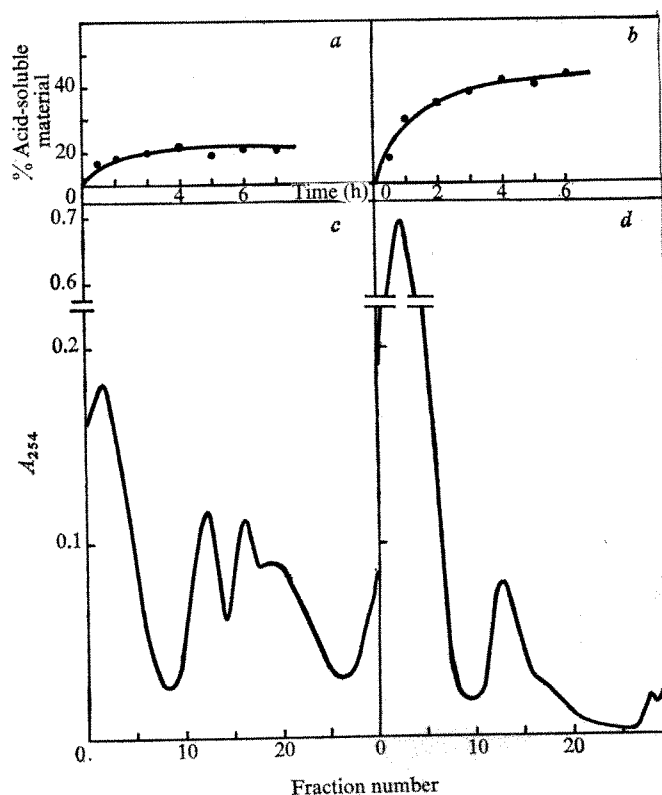


Fig. 4 Digestion of chromatin by DNase II. Mild digestion of chromatin from dry (a and c) and 72-h-old (b and d) embryos was carried out as described previously¹⁰. Each assay contained 5 A_{260} units of chromatin ml^{-1} , and DNase II at a concentration of 6.4 μg DNase per A_{260} unit. Aliquots were taken at various times to test for the production of TCA-soluble material (measured by absorbance of the supernatant at 260 nm after centrifugation at 27,000g and 4°C for 15 min). The kinetic studies of the hydrolysis (a, b) revealed a rapid initial digestion followed by a slower one. Sucrose gradient analyses (c, d) were carried out during this slower process with chromatin digested for 4 h. An aliquot (1 ml) of each digest was layered on to a 5–20% sucrose gradient. Centrifugation was for 10 h at 40,000 r.p.m. and 4°C in a Ti SW 41 Beckman Rotor. Gradients were eluted with an ISCO apparatus.

sponding to different stages of germination was hydrolysed with low concentrations of DNase II, and the digests were analysed on sucrose gradients¹⁰. Figure 4 shows two extreme situations, corresponding to dry and 72-h-old embryos. The time courses of the digestions are shown in the top panels. The profiles are similar to those obtained by other workers^{10,11}. The slowest sedimenting particle can be considered as the nucleosome monomer since it exhibits the following properties: it contains all histones except H1, a short DNA molecule homogeneous in size, and has a buoyant density in CsCl of 1.48 g cm^{-3} . Digestion of germinated embryo chromatin (Fig. 4d) yields a larger amount of hydrolysed material than dry embryos (Fig. 4c). These data have been extended to the overall period and the amount of hydrolysed material plotted against germination time (Fig. 5). Obviously the accessibility of DNA increases during germination and the changes correlate with a reduction in the histone/DNA ratio.

Our results provide original information on changes in chromatin structure in the course of a developmental process. They demonstrate that less and less histone is bound to DNA as the genome is re-activated and transcribed. Particularly relevant to the relaxation of the genome was the progressive disappearance of histone H1, the latter being assumed to help in condensing chromatin¹². Note that the level of H1 increased dramatically during seed maturation and thus probably contributes largely to the functional inactivation of the seed genome¹³.

Our results are also in accordance with the observation that H1 is absent or very much reduced in actively transcribed chromatin from animal cells¹⁰. The other four histones remain in equimolar amount and the nucleosome structure is conserved during the overall germination period. As a consequence, the decrease in the histone/DNA ratio and the increase in the DNA accessibility in chromatin can be best interpreted as a decrease in the nucleosome number along the DNA fibre. These changes are correlated

with active transcription of numerous genes¹⁴ and accumulation of non-histone chromosomal proteins¹⁵. This suggests that although nucleosomes are clearly associated with actively transcribed genes¹⁶⁻¹⁹, nucleosome density along the DNA fibre might be a crucial parameter in controlling gene expression. Such a dramatic change can probably be demonstrated only when cell division is required for differentiation and when a large number of genes have to be switched on or off. For example, recent data²⁰ indicate that the differentiation of the avian red blood cell is accompanied by a progressive packaging of more and more histones along the DNA until it is almost completely inactivated. We describe here the reverse situation, in that a repressed genome is reactivated.

In addition, we show that most of the changes involving histones take place at the time of DNA synthesis. This observation has been possible only because most of the cells of the embryo axis replicate their DNA twice with relatively good synchronisation. Note that our results bring direct evidence that when cells divide in a developing system the DNA-bound stock of histone and the chromatin structure and composition are not necessarily identical in mother and daughter cells. This probably constitutes part of the mechanism which allows new sets of genes to be switched on, being a key element in controlling development and cell differentiation.

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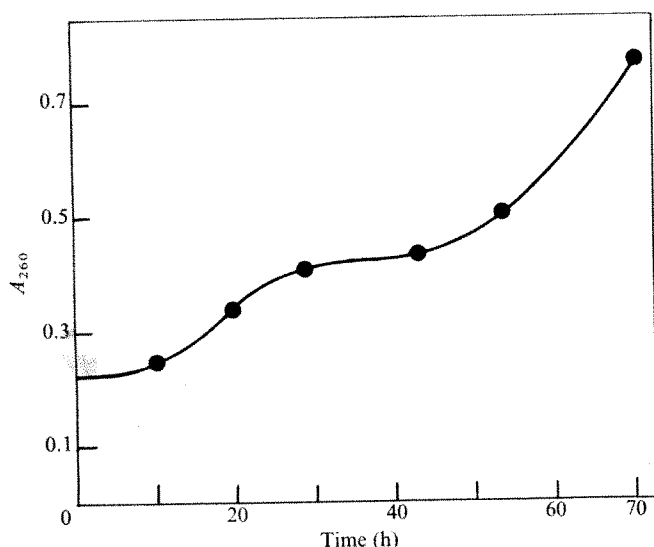
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Fig. 5 Change in DNA accessibility in chromatin during germination. At each stage of germination, the amount of material hydrolysed by DNase II was determined in a series of sucrose gradient experiments as described in Fig. 4c and d. The first seven fractions, corresponding to the absorbing material at the top of the gradient, were pooled and their A_{260} measured.



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matters arising

Circular superhelical DNA

BRADY *et al.*¹ have presented an excellent X-ray diffraction study of circular superhelical DNA and have shown that the native superhelix of circular DNA from PM2 bacteriophage produces two maxima at angles corresponding to Bragg spacings of 337 and 2,100 Å. We feel that the interpretation given by them for these maxima could be improved in several respects:

(1) The 337 Å maximum is due to superhelical formation by two DNA double helices. Brady *et al.*¹ have neglected the double nature of the superhelix so that their derivation of the

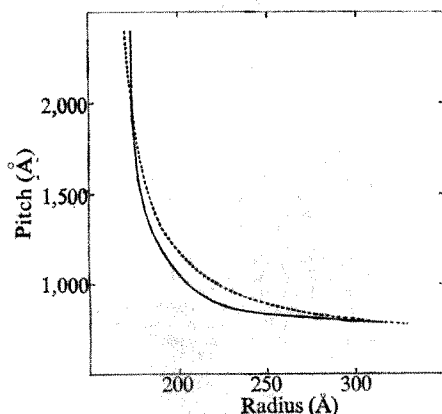


Fig. 1 Helical parameters (p , pitch; r , radius, in Å) for double superhelices which have a scattering maximum at 337 Å. The continuous line has been calculated as described in ref. 3. DNA was simulated by a cylinder of 20 Å diameter. The dashed line is the approximate equation (1) given in the text.

helical parameters is not correct. The first maximum observed will occur in the second layer line. This maximum is approximately related to the helical parameters through the relationship derived directly from the equation for ordered helices²

$$(1/d_{\text{obs}})^2 = (2/p)^2 + (0.487/r)^2 \quad (1)$$

A more accurate relationship can be obtained by numerical calculation from the scattering equation of disordered double helices³. Figure 1 shows that equation (1) describes with sufficient accuracy the relationship between the helical parameters p and r and the position d_{obs} of the first scattering

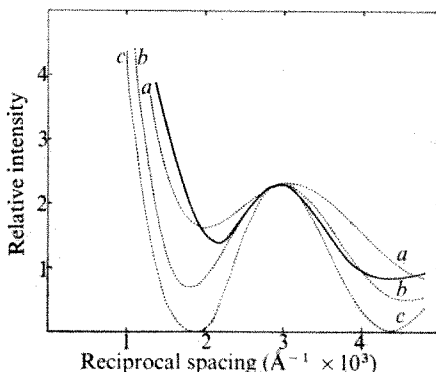


Fig. 2 A comparison of the experimental scattering curve of superhelical PM2 DNA (redrawn from Brady *et al.*¹) and three theoretical curves calculated by the method of Puigjaner and Subirana³: a , $p = 3,300$ Å, $r = 165$ Å; b , $p = 1,440$ Å, $r = 180$ Å; c , $p = 1,020$ Å, $r = 204$ Å. A very shallow maximum is found for large values of the pitch (curve a), since in this case the double superhelix approaches the behaviour of two parallel DNA molecules placed at a mutual distance of about 337 Å. On the other hand, for low values of the pitch ($p < 800$ Å, not shown) the double superhelix approaches a hollow cylinder and new maxima appear, which obscure the maximum due to the superhelical nature of the molecule. Taking into account that the experimental curve has neither been desmeared, nor the background corrected, the dimensions of the superhelix should fall between the values given for the b and c curves.

maximum. In order to determine the actual dimensions of the superhelix, use can be made of the fact that the sharpness of the maximum varies with p and r , as shown in Fig. 2. Unfortunately the experimental limitations¹ do not warrant an exact adjustment of the experimental and theoretical curves, but it can be concluded from such comparison that the radius of the superhelix lies in the range 180–200 Å, with a corresponding pitch in the range 1,400–1,000 Å, dimensions which are about twice those predicted by Brady *et al.*¹

(2) The peak at 2,100 Å has been attributed by Brady *et al.*¹ to a second-order superhelix. To determine its dimensions, Brady *et al.*¹ used an approximate formula which deviates significantly from the calculated values for single superhelices, as shown in Fig. 3. This is due to the fact that scattering in the equatorial plane interfaces strongly with the position of the first layer line peak. Furthermore, the 1,23 factor used by

these authors does not seem to be suitable to correct for the random orientation of helices. From the values shown in Fig. 3, and taking into account the shallow shape of the experimental maximum, it can be concluded that the secondary superhelix has a radius of 650 ± 150 Å and a pitch of $2,800 \pm 100$ Å. The pitch can be determined rather accurately, due to the maximum of the theoretical curve shown in Fig. 3. These values indicate that there are three to four second-order turns per molecule.

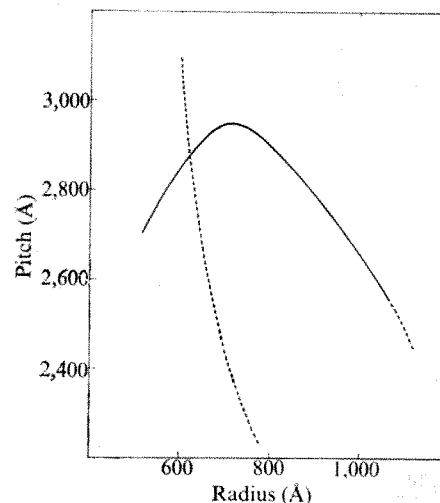


Fig. 3 Helical parameters (p = pitch, r = radius, in Å) for single superhelices which have a scattering maximum at 2,100 Å. The continuous line has been redrawn from the results presented elsewhere (Fig. 3 in ref. 3). The dashed line is the equation (1) used by Brady *et al.*¹. Calculation³ shows that the continuous line cannot be extended towards both ends, since the superhelical maximum is then obscured by diffraction in the equatorial plane of the superhelix. The sharpness of the maximum increases from left to right.

We should also mention, however, that other interpretations for the 2,100-Å peak are possible. It could be due, for example, to formation of a loop, such as a double superhelix, with the distance between the two strands close to 2,100 Å.

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BRADY *et al.*¹ have reported that the X-ray scattering pattern from superhelical PM2 DNA differs from that of its non-superhelical counterpart and interpret their results in terms of unusual tertiary conformational features of the molecule. In so doing they ignore the well documented chemical^{2,3} and enzymological^{4,5} evidence that the secondary structure of superhelical DNA is abnormal and fail to attempt any estimate of the quantitative effect that this may have on their experimental results. In addition, they have ignored the evidence from electron microscopy and light scattering which supports a branched structure for all superhelical DNA molecules with the regions of secondary structure variations situated at the end of the branches^{6,7}. As PM2 DNA possesses between four² and eight³ of such regions the concept of three high-order superhelices distributed over the entire molecule must be equated with there being less than one such turn per branch.

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Down's syndrome in Kerala

KOCHUPILLAI *et al.*¹ reported an abnormally high incidence of Down's syndrome in an area of high background radiation in coastal Kerala. They stated that the radiation exposure of their study group is 1,500–3,000 mrad yr⁻¹. But on the basis of a dosimetric survey² only about 11% of the females in their study received doses in the range of 1,100–2,000 mrad yr⁻¹ and 2.8% received more than 2,000 mrad yr⁻¹. This is due to non-homogeneous distribution of monazite-containing beach sands in the region.

Kochupillai *et al.* observed a frequency of cases of 1 : 1,076 in the study group. Comparison with any other published value is valid if the age structures of the two populations are similar. The infant mortality in this region is about 200 per thousand births³ and is independent of the high background radiation. Furthermore, in most other surveys only 4% of all births have been to females 40 yr old

and above. The data of Kochupillai *et al.* show about 20% of females were in this age group, which incidentally carries the highest risk of bearing children with Down's syndrome. Although in Sweden and Germany 27–29% of the population are in the age group 0–19 yr, in India more than 52% are in this age group³. The greater frequency of Down's syndrome in the study group of Kochupillai *et al.* than in those reported from other countries in ref. 1, could be explained solely in the differences in population structures.

Kochupillai *et al.* say: "Most striking is the higher frequency of cases of Down's syndrome born to mothers aged 30–39 yr (Table 3). This figure of 1 : 81 can be compared with 1 : 880 and 1 : 290 for maternal ages of 30–34 yr and 35–39 yr, respectively, calculated by Penrose and Smith⁷". But the ratio of 1 : 81 relates to the number of cases of Down's syndrome per female in the age group 30–39 yr, and the figures of Penrose and Smith⁷ are for frequencies among all births in these age groups. These are not comparable. Kochupillai *et al.* have demonstrated only that mothers in the age group 30–39 yr run a risk 10 times greater than those in the younger age group, which agrees with other evidence of increasing risk with advancing maternal age. They make no comment as to why this risk is about three times lower in mothers in the age group 40–49 yr. If high background radiation is involved, an even greater risk would be expected in this group than in comparable age groups of the general population. The age-specific risk increases almost exponentially from 1 in 290 at 35 yr to 1 in 46 in mothers aged 45 yr and older⁴.

The incidence of Down's syndrome at birth varies from 1 : 520 to 1 : 873 based on nine surveys⁴, with an average of 1 : 663 or 1.507 per 1,000 births for all age groups. Frequencies at birth of around 1 : 800 have been reported for several population groups in India⁵ which experienced normal background radiation. Lejeune⁶ compiled reports which demonstrated no difference between Oriental and Caucasian populations.

Based on values of family size (6.2), the number of females (2,213) in the study population (12,918), an infant mortality rate of 200 per thousand births and a mortality of 60 per 1,000 births in the age group 1–20 yr, a total of 11,500 births is estimated in the study population of Kochupillai *et al.* Using a mean value reported by Penrose and Smith⁷ of 1.507 per 1,000 births, about 17 cases of Down's syndrome would be expected at birth. On the basis of data for the maternal age at birth reported by Verma⁸ and Collmann and Stoller⁴, I have made a more

rigorous calculation, by redistributing the total births estimated into various age groups, on the basis of the number of females in each age group in Table 3 of ref. 1. This gives about 20 cases of Down's syndrome per 11,500 births. (The value should be even higher in this case because 20% of mothers were aged 40–49 yr, unlike any other populations studied.) This figure when corrected for mortality rate⁷ would leave 9 or 10 cases in the population at the time of the study.

Kochupillai *et al.* observed 12 surviving cases of Down's syndrome. The differences between expected and observed are not significant. On the same basis, one would estimate three surviving cases in their control population but none were observed. The differences are not significant. Although it is difficult to explain this, it could have arisen because of the smaller sample size used for the control population as against the study group, and the possible association of a major cardiovascular defect in these cases.

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KOCHUPILLAI *et al.*¹ reported that the prevalence of mental retardation of genetic origin was four times higher than normal in an area of high background radiation in coastal Kerala. As this background radiation is less than the limit of 5 rem yr⁻¹ recommended for registered radiation workers by the International Commission on Radiological Protection in 1965, this might cause anxiety both to those exposed and to those responsible for their welfare. We therefore wish to point out two reasons why the data are hardly sufficient to imply that the likely risks are so great.

First, 'severe mental retardation' is classified as a 'genetic abnormality'. Other data make it unlikely that more than a small proportion of such cases —of which mongolism (Down's syndrome) is the commonest—are due to any simple excess, deficiency or disturbance of the hereditary material.

Second, an area selected because mongolism was known to be common

was compared with an area in which no case was found in almost 6,000 persons, although one case would be expected in about 700 births.

The only firm data presented show that there were nine cases of mongolism born to 729 women aged 30–39 yr when about two would be expected—and two were born to women 40–49 yr old when about four would be expected. That is, 11 were observed, and, assuming no variation in racial incidence, about six were expected, making possible a very imprecise estimate of perhaps a doubling, perhaps more, but perhaps no increase.

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VERMA *et al.* REPLY—The differences in the age composition of the population in India and western countries, which are likely to affect the frequency of Down's syndrome are relatively small (Table 1). Infant and childhood mortality in India is about six times higher than in western countries¹. The mortality of Down's syndrome is expected to be substantially higher than in the general population. We believe therefore that the difference observed in the population frequency of Down's syndrome between the monazite area of Kerala and other countries² is not an artefact due to lack of standardisation for age structure.

We realise that our comparison of the number of cases of Down's syndrome born to women of specified ages with the frequency at birth may not be entirely valid³. Similarly, Sundaram's comparison of 4% of all births to females 40 yr old and above in other surveys with 20% of females in this age group is incorrect. The percentage of women 40–49 yr-old is less in Kerala than in western countries (Table 1).

Sundaram concedes that we demonstrated a ten times higher risk in women 30–39 yr old than in younger

women. This is more than twice the usually reported risk^{4,5}. The risk of Down's syndrome births in the 40–49-yr-old age group is no less than that in the 30–39-yr-old age group if the difference in age-specific fertility (about 56 and 186 per female in the two groups respectively)¹ is remembered.

Calculating the expected number of cases of Down's syndrome in the study population, Sundaram has incorrectly equated the mortality of Down's syndrome with the usual childhood mortality. It is more appropriate to estimate the rate at birth, starting with the observed number of cases: Down's syndrome patients, 12; less than 5 yr, 5; 5–10 yr, 4; more than 10 yr (average age 8.5 yr), 3. Assuming life table survival rates in cases of Down's syndrome in Kerala at 5, 10 and 20 yr as 40, 35 and 30% compared with Collman and Stoller's figures of 49.4, 46.2 and 38%, respectively⁶ (because of the substantially higher mortality in Kerala), the 12 observed cases would be survivors of 33 liveborn cases of Down's syndrome. Accepting Sundaram's estimate of 11,500 births, the frequency of Down's syndrome would be 2.8 per 1,000 births. This can be compared with a frequency per 1,000 births of 1.5 in the world⁴, 1.2 for an all-India series⁷ (excluding Madras), and 0.8 for Trivandrum, Kerala with normal background radiation (six cases of Down's syndrome among 7,167 births in 1976; personal communication from Saguna Bai).

With respect to the dose of radiation in the monazite belt, we have quoted only published figures^{8–10}. The area surveyed is recognised to have a peak level of radiation. Sundaram's group¹¹ has reported that 80.3% of households in this area recorded radiation exposure greater than 500 mrad yr⁻¹. Almost 27% of households had radiation exposure of 1,000–2,000 mrad yr⁻¹ (Fig. 5 of ref. 11). The dosimetric profile of individuals totally employed in this area is closely similar to the household exposure profile¹¹. With regard to the point raised by Edwards and Harnden, these doses exceed the safe genetic dose recommended by the ICRP for individuals (5 rem yr⁻¹) and for the general population (5 rem per generation but excluding natural background

and medical radiation)¹². The Advisory Committee on Biological Effects of Ionising Radiation sets the limit for the general population at 10 R before the mean age of reproduction of 30 yr (ref. 13).

Edwards and Harnden question our classifying 'severe mental retardation' as a genetic abnormality. This group comprised Noonan syndrome, primary microcephaly, mental retardation associated with craniofacial abnormalities and congenital blindness, and familial cases of mental retardation. No environmental factors were operative in these cases and we think it is reasonable to consider them to be genetic in origin.

The study area was not "selected because mongolism was known to be common". The area was surveyed because it has a high background radiation and a possible relationship with thyroid disease was being investigated¹⁴.

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Virus particles in marmoset hepatitis

SERUM from marmosets infected with the GB hepatitis agent has been reported (Almeida *et al.*¹) to contain aggregates of particles, 20–22 nm in diameter, and it was suggested that these particles may be the aetiological agent of GB hepatitis. Dienstag *et al.*² subsequently described the occurrence of 34–36-nm particles in homogenates of livers from marmosets infected with

Table 1 Age structure of population of females

Age group (yr)	Kerala ^{1,4} Study	Kerala ^{1,4} Control	India* 1961	Australia 1965	UK* 1966	Denmark* 1964
1–14	43.39	38.57	41.14	29.06	21.83	23.09
15–19	13.54	12.66	8.12	8.79	7.39	8.71
20–29	13.64	16.49	17.47	13.31	12.61	13.45
30–39	11.53	12.73	12.55	12.45	11.70	12.04
40–49	8.42	9.11	8.97	12.49	12.69	12.88
50 & <	9.48	10.42	11.75	23.90	33.78	29.83

Figures are percentages.

*Figures from ref. 15.

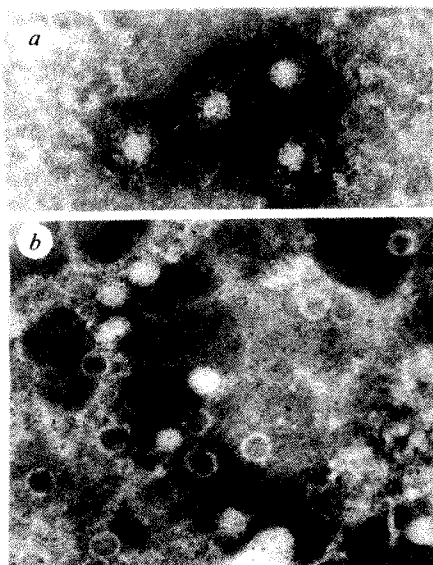


Fig. 1 *a*, 22-nm particles present in marmoset serum. These particles are apparently coated with antibody ($\times 168,540$). *b*, 22-24 nm particles found in marmoset faeces ($\times 168,540$).

the GB agent, but antibody to these particles could not be detected in convalescent serum from the patient (GB) himself. Findings from marmoset transmission studies in this laboratory support the suggestion that the small serum particles described by Almeida may be aetiologically associated with hepatitis in marmosets.

Serum from cotton top marmosets (*S.oedipus oedipomidas*) which had been infected with the Berlin agent which is antigenically related to GB contained small aggregates of round virus-like particles approximately 22 nm in diameter (Fig. 1*a*). These particles seemed to be heavily coated with antibody. Since this serum pool is highly infectious for marmosets presumably free virus particles exist, but electron microscopy is not a sufficiently sensitive technique to be able to detect these.

Similar 22-24-nm round virus-like particles were transiently excreted in the marmoset faeces. Particles were not found in specimens collected on the seventh and twelfth day after inoculation, but a stool specimen collected from one animal on the tenth day contained both full and empty particles (Fig. 1*b*). These particles did not occur in aggregates and there did not seem to be any antibody on them. Levels of serum transaminases were first found to be significantly raised in the serum sample collected on the eleventh day.

The occurrence of 20-24-nm particles in serum of marmosets infected with both the GB and Berlin hepatitis agents, and the transient excretion of similar particles in faeces at the time when levels of serum transaminases were elevated, suggests that these small

virus-like particles may have a significant role in the development of Berlin and GB hepatitis in marmosets.

Marmosets infected with the GB and Berlin agents did not develop antibody to the parvovirus-like antigen found in human sera by Cossart³.

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¹ Almeida, J. D. *et al.* *Nature* 261, 608-609 (1976).

² Dienstag, J. L., Wagner, J. A., Purcell, R. H., London, W. T. & Lorenz, D. E. *Nature* 264, 260-261 (1976).

³ Cossart, Y. E., Field, A. M., Cant, B. & Widdows, D. *Lancet* i, 72-73 (1975).

DIENSTAG REPLIES,—Appleton has demonstrated 22-nm virus-like particles in serum from *S. oedipus* marmosets infected with the Berlin agent, antigenically related to the GB agent¹. Like those in the report by Almeida

as presumed by Appleton, is highly unlikely. Whether these antibody-coated particles have been sought in non-infectious serum (pre-inoculation, convalescent, and/or normal marmoset serum) is not detailed in Dr Appleton's communication. Even if these antibody-coated particles cannot be detected in normal marmoset serum, as Almeida *et al.*² reported, conclusions about the aetiologic role of these particles are unwarranted, for the same reasons we pointed out in our letter⁴.

Regarding the detection of 22-24-nm particles in stool of one infected marmoset on day 10 but not day 7 or 12 after inoculation, the obvious questions are (1) whether pre-inoculation and convalescent stools were also examined, and (2) whether these particles can be aggregated by convalescent but not pre-inoculation serum from Berlin agent-infected marmosets. In our laboratory,

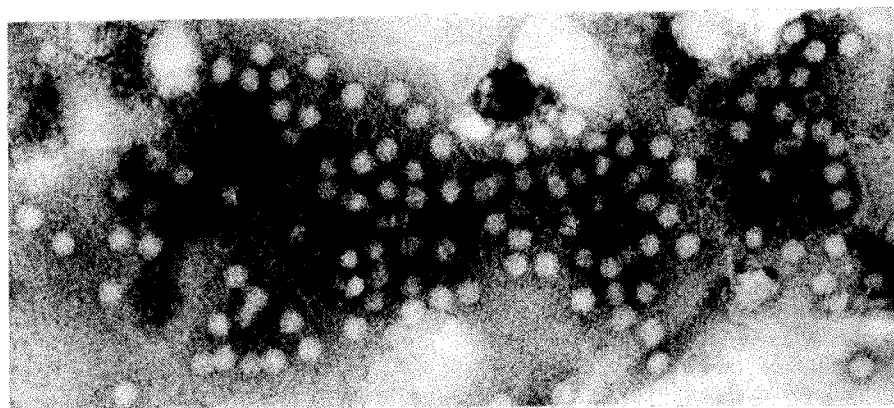


Fig. 1 Electron micrograph of 22-nm particles in the stool of a *S. mystax* marmoset. Such particles appear in normal marmoset stools as well as in stools of marmosets infected with hepatitis A virus and bear no serologic relationship to any known infection. (2% phosphotungstic acid negative stain) ($\times 132,000$).

*et al.*², the particles shown are heavily coated with antibody. Such a dense halo of antibody molecules and the large interparticle distance are characteristic of extreme antibody excess, rather than antigen excess³; therefore, the presence of antibody-free particles,

we have detected morphologically identical 22-nm particles (Fig. 1) in normal marmoset stools, in pre-inoculation, acute illness, and convalescent stools from marmosets infected with hepatitis A virus. These ubiquitous particles bear no serological relationship to any known illness and are easily distinguished from 27-nm hepatitis A antigen particles by immune electron microscopy⁵.

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Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

¹ Appleton, H. *J. infect. Dis.* 132, 500-505 (1975).

² Almeida, J. D. *et al.* *Nature* 261, 608-609 (1976).

³ Dienstag, J. L., Alling, D. W., Purcell, R. H. *Infect. Immun.* 13, 1209-1213 (1976).

⁴ Dienstag, J. L., Wagner, J., Purcell, R. H., London, W. T. & Lorenz, D. E. *Nature* 264, 260-261 (1976).

⁵ Feinstein, S. M., Kapikian, A. Z. & Purcell, R. H. *Science* 182, 1026-1028 (1973).

reviews

White beard through the looking glass

Richard Dawkins

Behind the Mirror: A Search for a Natural History of Human Knowledge. By Konrad Lorenz. Pp. 261 (Methuen: London, 1977.) £4.90.

A PURELY PERSONAL PREJUDICE leads me to appreciate a book more if I am told, at the outset, what it is the author hopes to teach me. In place of the introductory chapter which might have served this purpose, *Behind the Mirror* begins with 'Epistemological Prolegomena'—long on Kant but, apart from a cryptic last sentence, short on statements of intention. In the final chapter we learn of the author's aim to "attempt to give a survey of man's cognitive mechanisms", something that "no-one else has hitherto ventured to do". "Cognitive mechanisms" are interpreted liberally to include not only higher conscious faculties but also more rudimentary mechanisms of adaptive behaviour which might represent the evolutionary forerunners of consciousness.

The readership at which the book is aimed is also hard to pin down. It is certainly too cerebral for the mass audiences of *King Solomon's Ring* and *On Aggression*, although there are flashes of the humour and the eloquence that characterise those works. On the other hand, although there is a bibliography to match the undoubted erudition of the author, the text lacks the detailed citations which would encourage the professional scholar to follow up points of special interest.

Librarians should file it half way between Philosophy and Ethology, which is another way of saying that I am only half competent to review it. I lack the knowledge (to say nothing of the confidence) to make statements like "The only philosopher ever to voice similar opinions is the Austrian F. Decker". And I cannot professionally evaluate Lorenz's central philosophical point, which seems to me rather important, that "in the interests of objectivity a scientist must understand the physiological and psychological mechanisms by which experiences are conveyed to men... for the same reasons that a biologist must know his microscope...".

The early chapters include some of the arguments given in *Evolution and Modification of Behaviour* (1966). Certainly these points can bear repetition, having been much misunderstood by Lorenz's critics from the school of Hysterical Environmentalism. Animal behaviour is adapted to its environment. This means there is a sense in which facts about the

world are represented in the animal's nervous system or other parts of its physical structure. Just as a key embodies information about the lock which it is manufactured to fit, so even the simplest protozoan contains knowledge about its environment in the sense that it is equipped in advance to survive in that environment.

Those who have ranted against Lorenz's usage of "innate" have sometimes failed to appreciate that he applies the word not to behaviour itself but to the "adaptive information" of behaviour. This is vividly expressed in his treatment of learning: "Unless one believes in supernatural factors... one has to postulate the existence of innate teaching mechanisms in order to explain why the majority of learning processes serve to enhance the organism's fitness for survival. These mechanisms also meet the Kantian definition of the *a priori*: they were there before all learning, and must be there in order for learning to be possible".

In later chapters of *Behind the Mirror* Lorenz comes on to human culture. As always what he has to say is stimulating, but his comparison of biological and cultural evolution is marred by his unfortunate misconception about how evolution works, and therefore about the nature of adaptation. A junior ethologist is bound to be mindful of Lorenz's own emphasis, reiterated in *Behind the Mirror*, on the biological value of reverence for the ancestral wisdom of tribal elders. This is enough to arouse twinges of remorse over the tone of my own recent criticism of Lorenz's unconscious group selectionism. Perhaps it was unfair to pick on *On Aggression* which, after all, was first published in 1963, just before the reaction to Wynne-Edwards brought us back to our neo-Darwinian senses.

But it seems that nothing has changed. In *Behind the Mirror*, animals are still expected to behave for the good of the species, and

there is still no inkling that the issue is the slightest bit controversial. Lorenz sees a "balance between the factors that make for the invariance of the gene pool and those factors that make for its modification..." which "is adjusted to the degree of variability of the environment". Mutation rate is at its "highest in creatures that inhabit highly variable environments". This last statement fits Lorenz's world view like a glove, but it ruffles all my selfish-genetic hackles and I would have liked to have been told where I could look up the evidence.

The idea of a balance between mechanisms for and against change is developed in the analogy of cultural evolution, where it is thought to include genetically programmed tendencies on the part of the young to revere the wisdom of elders—the White Beard Effect—and at other times to rebel against it. Lorenz gives us some fascinating examples of the predominance of blind biology-like evolution rather than rational planning in the development of human culture, and he correctly identifies natural selection as the common factor. But his is a natural selection between large units—species in the biological case, civilisations in the case of culture—rather than, as it should be, natural selection *within* such large units. If you want a cultural analogue of the fundamental unit of biological natural selection, you should seek the analogue not of the gene pool but of something like the gene.

A reviewer is bound to mention the shortcomings he sees in a book, but in this case they are not new and therefore do not diminish his deep respect for the author's achievements, a respect which is far too solid and real to owe its origin to any White Beard Through the Looking Glass. □

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Language analogue project

Language Learning by a Chimpanzee: The LANA Project. Edited by D. M. Rumbaugh. Pp. 312. (Academic: New York and London, 1977.) \$17.50; £12.40.

LANA is a laboratory chimpanzee, born in October 1970, who has been learning a specially-designed 'language' and engaging in a number of related experiments at the Yerkes Primate Center of

Emory University, Atlanta, Georgia, since she was just over two years old. The 'language' that she is learning is called, appropriately enough, 'Yerkish'; and her own name is identical with the acronymic abbreviation of the formal title of the project; the LANA project is, in full, the Language Analogue project. With the publication of the present volume, which reports the results of the first three and a half years

work, Lana bids fair to becoming as famous as her conspecifics, Washoe and Sarah, trained by the Gardners and the Premacks, respectively. And deservedly so. The LANA project confirms some of the more interesting, not to say exciting, findings of the earlier research; and it makes its own methodological (and to a lesser extent, theoretical) contribution.

The book falls into five parts, consisting in total of sixteen chapters. There is also a very brief foreword by Karl H. Pribram, in which the point is made that "Washoe, Sarah and Lana—and a whole new generation of scientists and apes—are attempting to spell the end of Cartesian dualism". It is also suggested, rather misleadingly to my mind, that "a great deal can be learned about human language from a careful reading of the early chapters". Ernst von Glasersfeld's "Linguistic Communication: Theory and Definition" (chapter 2) does little to advance our understanding of either the structure or the functions of language. Richard K. Davenport's "Cross-Modal Perception: a Basis for Language?" (chapter 3) is no more than suggestive: it is an extremely important finding for comparative psychology that certain non-human primates are capable of cross-modal perception, but the implications of this finding in relation to theories of the nature and origin of language are not drawn in detail.

The most substantial contribution in part I is chapter 1, by Gordon W. Hewes, on the history and current state of speculation about the origin of language. Hewes (who is the only one of the fourteen contributors not to have worked on the LANA project and been responsible for aspects of its design, its day-to-day operation, or the running of some of the experiments) has probably done more than anyone else to resuscitate interest in glottogenic studies and, more particularly, to revitalise, in the light of recent work in several disciplines, the eminently plausible hypothesis that human language originated in gesture, rather than vocal signals. His chapter, as one would expect, is informed and judicious; although it is rather too sketchy in parts and suffers, in the present context, from being geared, as far as references are concerned, to his recent two-volume bibliography. It will no doubt, however, serve the purpose for which it is intended, that of putting the LANA project and other work in "the Chimpanzee Era of glottogenic research" (pp 38–42) in its historical and currently multidisciplinary context.

Lana's training has been more similar to Sarah's than to Washoe's, being based (although less strictly than Sarah's) on the principles of operant

conditioning. Yerkish, too, is like Sarah's plastic-token 'language' in that its grammatical structure and the form and meaning of its 'words' (combinations of up to nine readily discriminable line-figures superimposed on a coloured background) have been fixed in advance by its designers and are rigidly controlled. The syntax is very simple, although otherwise not strikingly different from that of many natural languages in the use that it makes of word order, sentence particles and various predicative relationships. There is provision for co-ordination and embedding. But so far this facility has not been used in the training programme. Nor have other complexities that it is planned eventually to build into the system. It is probably fair to say that Lana's progress with Yerkish is comparable with the progress that Washoe and Sarah made with their 'languages'. It is still an open question, therefore, and to some considerable extent a matter of definition, whether apes have been shown capable of learning a communication system that is rightly des-

cribed as a language. But the research is undeniably of great importance, and the results that have been obtained so far are challenging.

The principal methodological contribution of the LANA project comes from the total automation and computerisation of the process of recording and analysing all the strings of symbols, both by Lana herself and by her 'interlocutors', at all times of the day. As the contributors point out, this has considerable implications for the development of further hypotheses and the analysis of experimental results. The central chapters of the book provide a full account of both the software and the hardware, of the training programme, and of a variety of experiments carried out so far. Much of this has been reported already at conferences and in the specialist journals; but it is convenient to have it brought together in a single volume.

John Lyons

John Lyons is Professor of Linguistics at the University of Sussex, UK.

Pharmacology of the synapse

Chemical Pharmacology of the Synapse. By D. J. Triggle and C. R. Triggle. Pp. viii+654. (Academic: London and New York, 1976.) £20; \$43.75.

THE five chapters in this book cover the structure and function of the synapse (127 pages); quantitative aspects of ligand-receptor interactions (102 pages); structure-activity relationships: chemical constitution and biological activity (197 pages); the molecular basis of neurotransmitter-receptor interactions (163 pages), and characterisation, quantitation and isolation of neurotransmitter receptors (41 pages). It therefore attempts to cover biological and chemical topics which are of fundamental importance in the understanding of the actions of drugs at receptors.

The first chapter should be helpful as an introduction for chemists and biochemists. The second deals with the relation between dose and response in experiments where this is not complicated by metabolism and transport. Some pharmacologists may feel that the treatment is not as rigorous as it might be, but it is a fair account of current ideas. The third chapter reviews structure-activity relationships at eight separate receptors, and although this is the biggest chapter in the book and

contains much useful information, it is rather fragmentary. Each receptor really requires more extensive discussion and the non-chemical reader may find this chapter heavy going. It also needs some underlying idea to hold the eight sections together and an attempt to supply this is made in the fourth chapter. In our present state of knowledge, however, the molecular basis of neurotransmitter-receptor interactions is very uncertain and the chemist may feel disappointed when faced with an account that is inevitably largely electrophysiological. Understanding the basis is, in fact, going to require further information, which should come from studies with isolated receptors, dealt with briefly in the last chapter. The book, therefore, does not supply all the answers, but it gives a reasonable account of the problems and of the work which has been done to try to solve them.

There are some blemishes. The authors ought to make up their minds whether to use (*d*) or (+) for the sign of rotation and the formula for nicotine is twice given incorrectly (p22, p292); only half the absolute configuration is indicated in the formula of (+)-tubocurarine (p23).

Although most readers will probably find something to object to somewhere in the book, the authors have done a useful public service in collecting together so much information, much of it recent. The subject needs a book of this sort, which will make a useful starting point for students who are new to this kind of work, whether they are

chemists, biochemists, pharmacologists or physiologists. All these will find some part of the book where they will feel at home, and this should encourage them to see their contribution in relation to that of others. Although it may be difficult to write a book all of which will appeal to readers with such different backgrounds, the subject is

not divided up in the way that scientists are. This book is important because it attempts to review synaptic events as a whole.

R. B. Barlow

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Recognising variable patterns

Syntactic Pattern Recognition: Applications. Edited by K. S. Fu. Pp.xi+270. (Springer: Berlin and New York, 1977.) DM 85; \$37.40.

THIS collection of ten papers by different authors is concerned with the application of automatic pattern recognition to electrocardiograms, speech, Chinese ideograms, mathematical expressions, finger prints, remotely sensed imagery and parts of industrial assemblies. The central technical problem is that machines are required to recognise patterns that are not unique. For instance, countless minutely different waveforms should be recognised as clinically normal electrocardiograms. Similarly, two successive finger prints from one right thumb will generally be slightly different, depending for instance on distortion of the skin by pressure while the print is taken. Such differences often make it unexpectedly difficult to make machines recognise what the brain can easily recognise.

There is at least a tenuous analogy between deciding which patterns are *bona fide* finger prints of one right thumb and deciding which strings of symbols constitute a syntactically admissible sentence in a formal language. In both cases a large or infinite set of strings or patterns has to be defined by means of definitory apparatus that contains relatively little information. Furthermore, a finger print consists for instance of a whorl or tented arch which in turn consists of minutiae in various positions; and similarly a sentence consists of phrases which in turn consist of words. This illustrates the general idea that patterns, as well as sentences, can usefully be subject to a hierarchical structural description as in parsing. Because formal language theory is well understood, various workers have tried since about 1964 to do some good with it in automatic pattern recognition.

Chomskian grammars turn out to be

too abstract, too simple, for immediate application to pattern recognition. First, a phrase structure is concerned only with concatenation of symbols, not with the details of their relative positions and shapes. Second, a simple grammar is one-dimensional, whereas finger prints are not. Both of these problems can be overcome to some extent, and the book under review gives usually *ad hoc* details for the different practical applications.

The first chapter is a concise introduction by Fu, whose textbook *Syntactic Methods in Pattern Recognition* (Academic Press, 1974) makes an even better introduction. The standard of the remaining papers is rather higher than in the usual journals, and several contain useful introductions and bibliographies for their application areas. Stallings' paper on Chinese ideograms is particularly good in this respect.

In pattern recognition one can either start with a technique and see how it works in a given application, or one can start with an application and devise suitable techniques. The present book mainly follows the first of these strategies, whereas the papers in *Digital Picture Analysis*, Edited by A. Rosenfeld (Springer, 1976) mainly follow the second strategy and turn out *not* to be straightforwardly syntactic in the pure linguistic sense. The syntactic approach is just one among many; and it has perhaps met its greatest commercial success in reading machines such as the IBM 1287 which are not mentioned in the book under review.

This book is not about established commercial applications but instead reports research explorations of conceivable applications for syntactic techniques, and does not give much idea of the relative efficacy of syntactic compared with other techniques. Nevertheless, it is as good a collection of papers as we are likely to get in 1977 on this topic.

J. R. Ullmann

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Foundations of population genetics

Foundations of Mathematical Genetics. By A. W. F. Edwards. Pp. viii+119. (Cambridge University: Cambridge, London and New York, 1977.) £5.80.

JUDGING from the title, you might expect this book to be a history of population genetics. It is a fine book; but it is not a history, certainly not in the conventional sense. The book discusses the foundations of population genetics, and to A. W. F. Edwards that mainly means the single locus, infinite population, discrete generation, random mating, constant fitness model. There is some justification for focusing on this model and closely related ones, for, as Edwards points out, "... the elementary mathematical theory [of population genetics] has reached a sufficient maturity to be presented... as a piece of mathematical standing in its own right" (page vii). The approach is purely mathematical, therefore, and the book will be useful to mathematicians and statisticians who wish to have a concise introduction to the essential terminology and concepts of population genetics. It will also be useful to population geneticists, evolutionary biologists, or ecologists who want a thorough review of some of the fundamental models of population genetics.

In elaborating in detail the implications of a few fundamental models, Edwards goes somewhat counter to what has been and remains an historical trend in population genetics, a trend toward generality at the expense of precision. How this trend became established is not completely clear, but Edwards attributes it to the genius of the founders: "One of the inevitable corollaries of the fact that the structure of mathematical genetics was created by three men of exceptional calibre, R. A. Fisher, J. B. S. Haldane and S. Wright, has been that the ground floor of the edifice is scantily furnished. They ascended rapidly to the upper storeys, not always by the same staircase" (page vii).

On the other hand, the quest for generality has characterised not only population genetics but also much of theoretical evolutionary biology. Emphasis has been on the search for principles that apply at all times and to all situations; special cases, or principles with a restricted range of validity, are anathema. What we have learned in the past fifty years is that there are precious few general principles, and these few are so abstract as to be not especially useful.

Edwards has in any event countered the trend towards a superficial analysis of a multitude of models. This seems thoroughly appropriate for a book whose purpose is to present elementary population genetics as a branch of mathematics. Whether the approach is equally valid for those whose interest is in actual, evolving populations is another matter.

In focusing on a few specific models, Edwards has discovered that, as the creators of population genetics ascended to the upper storeys, they left a number of important theoretical gaps below, in the analysis of the simple, fundamental model of population genetics. In a number of instances Edwards has plugged the gaps himself, so the book contains much that is new.

The book focuses on the discrete-generation models of selection in diallelic, triallelic, and multiple allelic autosomal loci as well as sex-linked loci; brief sections are also devoted to models with differential viabilities in

the sexes, selection in heterogeneous environments, and selection with two linked, diallelic autosomal loci. Throughout the book, emphasis is on finding conditions for the existence of polymorphic equilibria and determining when the gene frequency will actually converge to the equilibrium. Considerable attention is also given to the behaviour of the mean fitness function. One attractive feature about the book is a consistent and clever use of triangular coordinates, following a century-old treatise on the subject by Reverend N. M. Ferrers, one of Edwards' predecessors as Fellow and Tutor of Gonville and Caius College.

All in all, *Foundations of Population Genetics* is a thorough, scholarly treatise, and a useful addition to any professional's library.

Daniel L. Hartl

Daniel L. Hartl is Professor of Biology at Purdue University, West Lafayette, Indiana.

Molluscan atlas

Atlas of the Non-Marine Mollusca of the British Isles. Edited by M. P. Kerney. Pp. v+202. (Conchological Society of Great Britain and Ireland: Luton, UK, 1976.) £3.

It is ironical that a major contribution to the study of land and freshwater molluscs in the British Isles should have been published in a year when a catastrophic drought produced a major perturbation on many molluscan populations. The production of this atlas is therefore apposite in providing basis for monitoring ensuing changes. Moreover, it commemorates a century of cooperative endeavour in recording the distribution of molluscs by a succession of dedicated enthusiasts.

This volume deserves recognition by a much wider audience than malacologists, for it summarises in graphical form information collected during the most detailed survey undertaken in this country on any invertebrate group of comparable size. Indeed, it represents a milestone in the development of mapping schemes, and should provide a catalyst to other small organisations undertaking similar projects.

The distribution of 192 taxa is presented on the now conventional 10-km grid maps used by the Biological Records Centre and the information is categorised on the basis of recorded living before or after 1960.

A further refinement is included: the records of the relevant fossils are plotted for those species where there is evidence of changes in the distribution since the last Postglacial period. A promised set of transparent overlays will provide information on a range of environmental parameters and thus enhance the interpretation of the maps, these are eagerly awaited.

Results have already accrued from this survey and these are reflected in the recording of several species new to the British Isles and in the re-assessment of the distribution or taxonomic status of others. Nevertheless, the enduring value of this type of work is the enormous impetus and stimulus it should provide for a wide range of research programmes and other activities.

Many biologists and naturalists will regret the adoption of a number of unfamiliar innovations in nomenclature even though they will applaud the desire to establish uniformity of treatment throughout Europe. This laudable objective would have been more palatable by the addition, to each map, of references to more widely known synonyms. An indication of the habitat range and extra-territorial distribution of each species would have further facilitated an understanding of the recorded patterns. Such limitations, however, should not detract from the value of this atlas.

J. F. Peake

J. F. Peake is Head of the Mollusca Section and Deputy Keeper of Zoology at the British Museum (Natural History).

obituary

L. F. Cooling

DR L. F. COOLING died on Tuesday, 15 February after a short illness. He was 73. His death marks the close of a significant era in the development of engineering science in Britain. Born in Birmingham, he graduated there in physics in 1925 and later gained an MSc for studies of the thermo-magnetic properties of meteorites. In 1927 he joined the Building Research Station at Garston and he remained there until his retirement as an assistant director 41 years later in 1968.

His early work on permeability and evaporation properties of porous building materials formed a good basis for his future research into the engineering properties of the ground. In 1933 he was made head of a soil physics section formed to carry on Prof C. F. Jenkin's work on earth pressure and to study road foundation problems for the Road Research Board. His laboratory in the Stable Block at Garston was the pioneer soil mechanics laboratory in Britain.

Cooling was the only British participant at the first International Soil Mechanics Conference in Harvard in 1936 where he acquired confidence in

his subject. By 1937, when he had been joined by Prof A. W. Skempton, FRS, and Dr H. Q. Golder, he had aroused considerable interest among civil engineers in the practical value of soil mechanics by his investigations of several landslips involving railway cuttings and retaining walls. In particular his diagnosis of the Chingford Reservoir embankment failure, which brought Karl Terzaghi to England for the first time, provided the impetus for the recognition of soil mechanics as an engineering discipline throughout Britain and engineers from many organisations came to the Building Research Station to be trained.

Cooling was practically minded but he never pretended to be an engineer. His lucid understanding of physics enabled him to convince engineers of his reasoning and they had absolute confidence in him. He was unpretentious and always talked sense.

During the war years the emphasis on the investigation and analysis of foundation and earthwork failures grew. The field studies pointed to weakness in the understanding of the in-situ mechanical behaviour of the ground and Cooling initiated a programme of intensive field investigations in which

detailed measurements were made of the performance of structures such as earth dams, tunnels and building foundations.

Cooling's contributions to engineering science were recognised in 1952 by his University with the award of a DSc, this time in engineering, and by the Institution of Civil Engineers who invited him to their membership. In later life Dr Cooling played many leading roles in the committees, societies and journals concerned with the national and international organisation of soil mechanics. He gave the second Rankine Lecture "Field measurements in Soil Mechanics" to the British Geotechnical Society who also recognised his interests in young people by creating the Cooling Prize, to be awarded annually to the best presentation from a young engineer—a function he always looked forward to in his retirement.

Cooling was a keen sportsman. At University he played for the England Amateur football team. He was a Midland Counties Champion runner and an enthusiastic player of shove ha-penny and golf.

He leaves a wife and a daughter.

W. H. Ward

E. Cunningham

Ebenezer Cunningham who died in Corbridge, Northumberland, on February 12 at the age of 95 was a pioneer in introducing the work of Einstein in this country. His father was a cabinet maker; G. D. Cunningham, organist and conductor, was his elder brother. From Owen's School, Islington, he won a mathematical scholarship at St John's College, Cambridge, was Senior Wrangler in 1902 and a Smith's Prize-man in 1904, when he was elected to a Fellowship. After holding lectureships at Liverpool University and University College, London, he returned to St John's in 1911 and until 1976 his home was in Cambridge, where he played an important part both in the Faculty of Mathematics and in his college. His clear and stimulating lectures on branches of applied mathematics are remembered with pleasure and a pupil says of his approach to an unfamiliar

problem, "you were seeing the Senior Wrangler in action."

His first few papers were on pure mathematics but by 1907 he was writing on electromagnetism; in 1909–12 he and H. Bateman, for a time a colleague at Liverpool, contributed papers to the *Proceedings of the London Mathematical Society* on the four-dimensional transformations of the electrodynamical equations. In 1910 he also made a correction to the Stokes formula for the velocity of steady fall of particles in a fluid.

For the next decade his main interest was the study and exposition of Einstein's theories. *The Principle of Relativity* (C.U.P. 1914) gave the first account in English of the Special Theory of Relativity. In 1915 he published *Relativity and the Electron Theory* (Longmans, Green & Co.) and in the second edition (1921) he included an introduction to the General Theory; "Gravitation" was added to the title.

His books emphasise comparison with theory at every stage; in most other accounts it is difficult to see how much of the theory is directly required by experimental evidence and how much, apparently, by pure thought. In 1919, very soon after the announcement on November 6 of the results of the Solar Eclipse Expeditions, he wrote three articles in *Nature* (Vol. 104) on "Einstein's Relativity Theory of Gravitation." In 1921 *Nature* for February 17 was devoted to articles on Relativity and his contribution follows that of Einstein.

He and his wife, whom he married in 1908, were active members of Emmanuel Congregational Church and they made many friends in all walks of life. He bore with courage her loss in 1969 and his increasing blindness. In this he was helped by his strong religious belief and his lifelong love of music.

Harold Jeffreys

G. M. Frank

ACADEMICIAN GLEB MIKHAILOVICH FRANK, the eminent Soviet biophysicist died on October 10, 1976.

Born in 1904, Frank graduated from the University of Simferopol in 1925. From 1929 to 1943, he worked at the Leningrad Physico-Technical Institute and then at the All-Union Institute of Experimental Medicine. From 1943–52 he carried out research in various laboratories of the Academy of Sciences of the USSR. During this time, he became increasingly associated with the Institute of Biophysics of the Academy of Sciences, becoming its Deputy Director in 1952 and Director in 1957. He was elected to the Academy of Sciences of the USSR in 1966.

Frank's research dealt mainly with the effect of ultraviolet light and ionising irradiation on animal organisms, and also the biophysical causes of nervous stimulation and muscular contractions. He made numerous innovations in photobiology, radiobiology and the biophysics of elementary life processes. He demonstrated the difference

in the biological effects of short-wave and long-wave ultraviolet radiation, showing that, in the first case photodenaturation predominates, and in the second photolysis. His work on ultraviolet radiation found numerous applications in Soviet medical practice, including ultraviolet anaesthesia and the treatment of traumas of the peripheral nervous system.

Frank played a leading part in the development of *in vivo* studies of early physico-chemical and functional changes in tissues exposed to ionising radiation. He identified a number of phenomena occurring during irradiation, and developing in the latent post-irradiation period (depression, variations in the oxygen utilisation pattern, changes in the erythrocytes, and in the electrical parameters of the tissues). He also developed the concept of negative feedback in cellular processes.

In 1927, Frank published a paper showing longitudinal sections of muscle fixed at rest and during isometric contraction at various initial lengths. He obtained the striking result that in con-

tracting muscle, the width of the A bands was independent of muscle length. This was a foreshadowing of the results which in 1953–54 contributed to the establishment of the sliding-filament theory of muscle contraction. His continued interest in this area was seen when he attended the Discussion Meeting on Muscular Contraction at the Royal Society in November 1963.

His major works include *Approaches to the in vivo investigation of the physico-chemical processes of nervous activity* (1954), *Early reactions of the organism to irradiation in dependence on local perturbations* (1955), and *Physico-chemical and structural principles of biological processes* (1960). He was co-editor of the radio-biology section of the second edition of the Soviet *Large Medical Encyclopaedia*.

For his services to Soviet Science and Medicine, Frank was twice awarded the Order of Lenin (the highest civil award) and four times the Order of the Red Banner of Labour. His research twice received a Stalin (now State) Prize. *Vera Rich*

Sheina Marshall

DR SHEINA M. MARSHALL, OBE, FRS, died at Millport on 7 April 1977.

After holding a Carnegie Scholarship at Glasgow University, during which she showed her research abilities in a study of the behaviour and structure of *Hydra*, she joined the staff of the Scottish Marine Biological Association's laboratory at Millport in 1922. Here she started immediately in the field of research in which she was to be engaged for the rest of her life with the most detailed study yet made on the feeding of the copepod *Calanus* and a description of a new species of dinoflagellate.

She then began a remarkable collaboration with A. P. Orr, chemist on the Millport staff, through which they became known internationally as pioneers in the study of marine production. Starting with experiments on the photosynthesis of diatom cultures in bottles submerged in the sea, they proceeded to their classic investigation of the seasonal changes in nutrients and phytoplankton in Loch Striven. In this work there can be seen the seeds from which much of the modern research on productivity has developed.

They then turned their attention to the biology of *Calanus* and other smaller copepods which form a major constituent of the zooplankton responsible for secondary production. In part

of the work on *Calanus* they were joined by A. G. Nicholls. A full study was made at sea of the general biology of these copepods, including their vertical distribution, successive generations throughout the year, their methods of feeding and chemical composition. This was later supplemented by experimental observation in the laboratory on respiration, food requirements, and excretion both in the adult and all young stages. By the use of radioactive phosphorus they showed its very rapid transfer to the ovaries and developing eggs and embryos, thus confirming their theory that abundant food is necessary for egg laying resulting in an adaptation whereby the hatching of the nauplii coincides with diatom outbursts.

After the death of Orr in 1962 Sheina Marshall started further collaboration with E. D. S. Corner, biochemist at the Plymouth laboratory of the Marine Biological Association. Thus her remarkable expertise and knowledge of copepod biology has been made available to a new generation of workers in the field of marine production. Her last investigation was concerned with the overwintering generation of *Calanus* in which it was shown that a partially carnivorous diet is probably necessary for survival.

In 1928 Marshall and Orr went with the Great Barrier Reef Expedition to Australia. There they undertook a similar seasonal investigation to that

made in Loch Striven on the nutrient and phytoplankton cycles in the reef area. This was the first to be done on this scale in tropical waters. Sheina, always indefatigable, made observations also on the occurrence of symbiotic algae in coral planulae, and with Orr investigated the ability of certain corals to rid themselves of silt.

On their return to Millport they made a short investigation, with A. G. Nicholls, on the growth of the early stages of the herring in the Clyde Sea Area, which is probably the most complete observation made on a single brood.

During the war she was engaged on two projects in the applied field, namely the fertilisation of sea lochs and the production of agar from the seaweed *Gigartina*.

Although not a systematist she had a special interest in tintinnids on which she wrote a Barrier Reef report and prepared plankton identification sheets for the International Council for the Exploration of the Sea.

Her work with Orr was brought together in their book *The Biology of a Copepod*, and she amplified and prepared for press Orr's Buckland lectures given in 1957 to form the book *The Fertile Sea*.

Much travelled, her loss will be felt by many friends all over the world who got to know her sterling qualities.

F. S. Russell

what's new – microscopes

In addition to the regular Newly on the Market section in *Nature*, special features on a particular type of laboratory equipment or instrumentation will be published in this and forthcoming issues. The issue of 18 August will be on Chromatographs and ancillary equipment and that of 20 October on Spectrometers and ancillary equipment.

The purpose of a special feature will not be to give listings of every item in the range of the manufacturers mentioned but to take a variety of equipment of interest in that feature area and give an idea of what kinds of instruments are available and from whom.

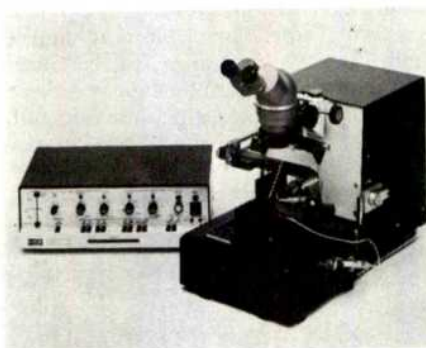
This feature on Microscopes and ancillary equipment covers optical and electron microscopes, stereomicroscopes, wide-field and measuring microscopes, teaching microscopes, stages, ultratomes and cryomicrotomes, fluorescence and metallurgical microscopes and other related items.

Available from Philips is a high-performance scanning electron microscope PSEM 500 interfaced with a fully-focusing wavelength dispersive X-ray spectrometer, the PSEM 500X, not only permits analysis of X-rays from $Z=5$ to $Z=92$ but is the first such system to use the vertical spectrometer orientation—a configuration which offers the highest mechanical reproducibility—without the need for an optical microscope. Instead, a new standard in specimen height accuracy and reproducibility is achieved via the unique, fully-eucentric goniometer coupled with its 4.6 mm fine Z-adjustment (in 0.2 μm steps) and the large (210 mm) Rowland circle of the spectrometer. An extremely high-vacuum system (10^{-6} torr, inclusive of the spectrometer assembly) vastly improves practical light-element detection. Thus, absorption of 'soft' X-rays from light elements, due to specimen contamination, is seldom encountered. The same vacuum system, without modification, allows operation with optional higher-brightness sources—a particularly valuable facility for excitation of X-rays from light elements. The standard 50 kV electron optics of the PSEM 500 is ideal, when using an energy dispersive X-ray detection system, for unambiguous excitation of K-line X-rays from heavier elements.

The larger excited volume of X-rays, when using 50 kV electrons, can also facilitate easier trace-element detection. The energy dispersive and wavelength dispersive spectrometers can be in operation at the same time, thus giving an optimum choice of the characteristics of each type of spectrometer.

Circle No. 45 on Reader Enquiry Card

LKB Instruments Ltd present an advanced ultramicrotome and a new 'whole animal' microtome/cryomicrotome. The Ultratome IV is the latest, most advanced ultramicrotome developed and manufactured by LKB-Produkter in Sweden. It will prepare large-area thick epoxy sections for scanning transmission or high voltage electron microscopy; and serial ultrathin sections for transmission electron microscopy. A system of push buttons allows knife alignment and approach to the specimen to be conducted entirely from the control panel. The gravity-powered cutting stroke ensures constant and reproducible cutting speeds regardless of section size or



LKB Ultratome IV

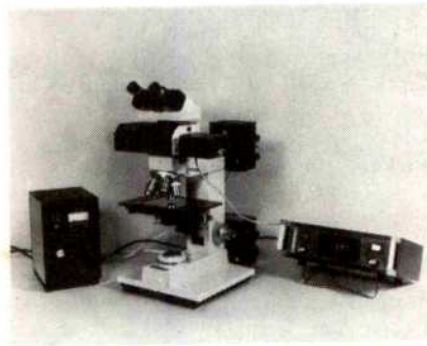
thickness, and this, in conjunction with the magnetic coarse feed, will prepare large, even sections for optical microscopy. Likewise, when used with fine thermal feed, the Ultratome will produce grid-sized sections to any thinness required between 0 and 200 nm. Other advances include specimen trimming and a new viewing system for selected area trimming; the orientation head offers precision of adjustment and provides wide specimen orientation possibilities. Ten glass knives can be evaluated at one time for edge quality and angle. There are new collection facilities for both thick and ultra-thin

sections. The PMV Cryomicrotome (made for LKB by Palmstiernas Mekanismiska Verkstad, Stockholm) is a heavy-duty microtome in a cryocabinet. Specimen size need no longer be a limitation with the PMV since material of up to 45 cm $\times$ 15 cm can be sectioned. Therefore experiments can be carried out using larger animals, a feature which could provide obvious advantages in the testing of new pharmaceutical products for eventual human use. Simple operation giving reliable and reproducible results identifies the PMV approach.

Circle No. 46 on Reader Enquiry Card

Vickers Instruments, a division of Vickers Limited, manufacture a comprehensive range of optical microscopes supplemented by selected instruments from a sister Company, Nachet of Franche, and from Union Optical of Tokyo. The Company also acts as sole UK agent for Metallographic and Mineralogical specimen preparation equipment by Struers of Copenhagen. Many recent innovations include an inexpensive closed-circuit television attachment suitable for routine industrial applications as well as the more traditional teaching role; a direct-reading micro-measurement system based on the image-shearing principle and capable of sub-micrometre accuracy even in inexperienced hands; the Nachet NS400 Research microscope featuring an optical system which allows all techniques to be undertaken using a single set of objectives, and the Union Versamet, an inverted Metallographic instrument allowing instant selection of bright field, polarisation, dark ground or Nomarski interference contrast. The most significant development has, however, been the introduction of a completely new series of instruments, collectively titled M17, for research and routine applications in both Materials and Life Sciences. The M17 series is designed to accept many interchangeable accessories but the availability of several basic microscope stands allows the user to select precisely those facilities which are required. The basic range already includes purpose-built models for incident light, transmitted light and dual illumination (one offering an extra-wide 25 mm field of view) while a totally

new incident fluorescence illuminator combines simplicity of operation with true research capability. A routine fluorescence microscope is also available and instruments for analytical work in polarised light are to be released soon. The flat-field objectives, both achromats and apochromats, are completely new designs, giving improved colour corrections over the widest 25 mm field of view. A comprehensive range of fully interchangeable camera equipment, including electronic exposure units, may be fitted to any model from the M17 series; particular features of the system being the retention of full binocular vision during exposure, the absence of the usual auxiliary focusing adjustment and the facility to rotate both framing graticule and camera body to capture oblique features of the specimen.



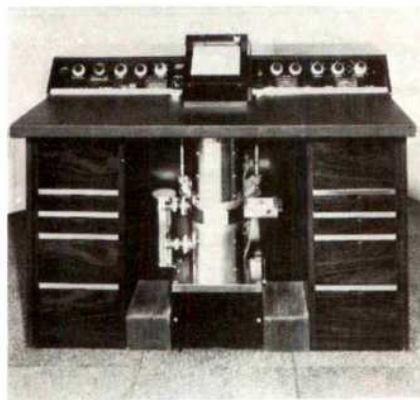
Vickers M17

Circle No. 47 on Reader Enquiry Card

Gillett & Silbert produce a broad range of microscopes from the student to the research level with the emphasis on those used routinely in the laboratory; the range includes photographic systems and a £100,000 consignment was recently delivered to Korea.

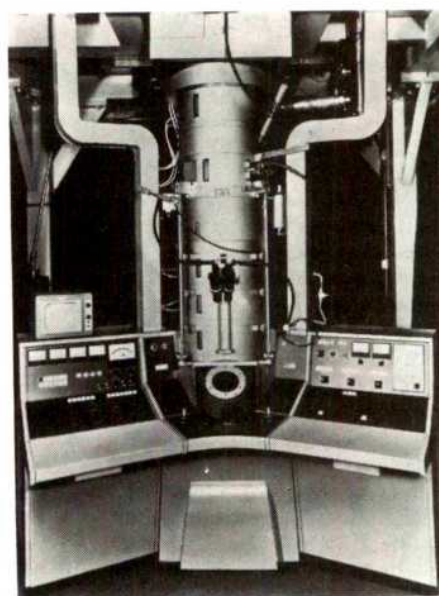
Circle No. 48 on Reader Enquiry Card

Kratos Ltd, AEI Scientific Instruments (formerly AEI Scientific Instruments) are long-time manufacturers of electron microscopes, two of which are featured here. The Corinth 500 is a high resolution electron microscope with 5 Å resolution imaging, multiple specimen facilities and multiple accelerating voltages of 20, 40, 60 and 80 kV. A double condenser lens system reduces specimen damage; there are excellent viewing facilities— $\times 240,000$ top magnification and excellent recording facilities—70 mm roll film camera is standard equipment. The Corinth has very high stability supplies— 5×10^{-6} per min and a foolproof fully automatic vacuum system. Also X-ray leakage rate is very low—less than 0.1 mR h^{-1} . Additional optional extras include viewing hood for daylight working, scanning attachment for reflection scanning, electron imaging of larger specimens,



AEI Corinth 500

solid state detector attachment for simultaneous transmission imaging and X-ray analysis of selected areas. Because of the high penetration of the electron beam, much thicker specimens can be investigated in a high-voltage electron microscope than in a 'conventional' instrument operating with an accelerating voltage of 100 kV. For this reason in particular, metallurgists and material scientists in the 1960s were the first to take advantage of the high-voltage technique. The thicker specimens which could be used were more representative of the bulk material from which they were prepared and they consequently revealed information about the material which could not be obtained by any other means. The high-voltage microscope also provides the biologist with facilities to exploit the exciting technique of stereoscopic micrographs. The thick sections of biological material make ideal subjects for viewing and photographing from two different angles, and, for the first time, it has been possible to record effective three-dimensional images which provide quite remarkable information and a new

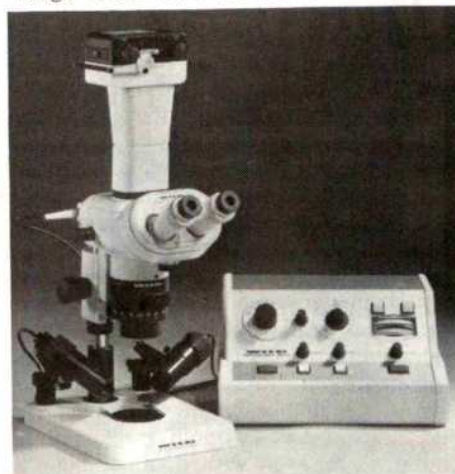


AEI EM7 Mk II

understanding of structure. One of the most recent applications of the high-voltage microscope is in geological research. Using specimens prepared by the ion beam technique, only a small part of the thinned section can be investigated with conventional electron microscopes. But, with a million-volt instrument, information can be obtained from a far greater area of the section which is more representative of the bulk material and the advantages of the technique hold promise for many new far-reaching discoveries in this field. The EM7 Mk II high-voltage electron microscope has a 5 Å resolution, a wide operating voltage range—100–1250 kV, can be used for critical voltage experiments and radiation damage experiments. It has wide magnification range— $\times 63$ to $\times 1,250,000$ and is suitable for grid searching and high resolution applications. Rotation free imaging— $\times 4000$ to $\times 1,000,000$ enables an area to be viewed with zoom magnification without the image rotating. Operation is very easy, all controls are conveniently placed, specimen chamber is within easy reach of operator. There are excellent viewing and recording facilities and accessories available are solid-state elemental analyser, closed-circuit TV system, image intensifier, radiation monitors, plate desiccator, liquid helium anticontaminator, high resolution diffraction stage, vacuum coating unit.

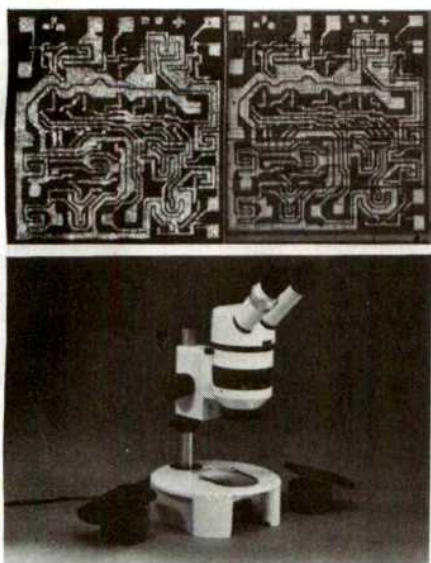
Circle No. 49 on Reader Enquiry Card

Wild Heerbrugg Ltd, Switzerland, present a photomicroscope, the Wild M400. This system opens up even to the non-specialist the hitherto rather difficult photographic magnification range from 1:1 to 60:1. Binocular



Wild M400

observation, zoom optics 1:5 and completely automatic exposure and film transport allow the observer to concentrate entirely on the specimen and format limits. Owing to its modular construction with different stands and other accessories for incident light,



Wild M5A

transmitted light, bright field and dark field, polarisation etc, the Wild M400 can meet all the demands of present-day macroscopy. The new Wild M5A Stereomicroscope has several new design features, particularly the availability of a unique new coaxial incident illuminator which directs the light of a 20 W halogen illuminator along the two optical trains, with the result that the image is free from the glare which is often so troublesome in the examination of highly-reflecting metallurgical specimens or of multilayer thin-film materials such as integrated electronics circuits. Additional objectives and interchangeable eyepieces extend the magnification range to $\times 250$. A metal hood, which unites with the baseplate, protects the instrument from dirt and from transport damage. The modular design enables a variety of observation techniques and supplementary sets of accessories (for example for photomicrography and television microscopy) to be applied.

Circle No. 50 on Reader Enquiry Card

Available from **Stanton Redcroft** is a hot stage microscope unit, the HSM-5, a unit which enables the user to observe materials under controlled conditions over the temperature range ambient to 1000°C . The unit may be used with any large working distance microscope especially with a stereoscopic microscope. 35 mm photomicrography and time lapse and high speed cine photography are readily possible. The sample temperature and the intensity of the light reflected from the sample can be accurately followed. The sample is held in a standard DTA sample pan. Specification: water-cooled micro-furnace with temperature range ambient to 1000°C . Rapid temperature response and cooling from

1000°C to below 100°C in less than 5 min; wide range of switch selected linear heating (and cooling) rates from 1 to 100°C per min. Rapid establishment of isotherms to within $\pm 1^\circ\text{C}$. Full atmosphere control, small swept volume for ready coupling to a gas analysis system. Accurate sample temperature measurement using plate type Pt v Pt-13% Rh thermocouple. No special sample preparation necessary. Quartz window for viewing. Can be rotated to remove condensed volatiles from the field of view. The unit has been used to study a range of reactions including the fusion and decomposition of organic and inorganic materials, oxidation of metal powders, the behaviour on ignition of pyrotechnic mixtures.

Circle No. 51 on Reader Enquiry Card

Bausch & Lomb UK Ltd market a wide variety of microscopes, stereomicroscopes and stereozoom microscopes for use in education, industry, electronics, R&D, medicine and the Laboratory. The ESM 25/100 Microscope is ideal for elementary science teaching, as well as a highly practical instrument for field observations. It combines two magnifications in one instrument, the $\times 25$ position for low power scanning and for macro specimens up to 10 mm thick, the $\times 100$ position for higher power detailed study. Model SSM Student Stereomicroscopes ($\times 15$) and Model ST2 Science Teaching Zoomscopes ($\times 50$ to $\times 200$) are both low-priced durable student microscopes. The Academic Stereozoom series of microscopes are also designed for science teaching, with zoom optics, 'student proof' construction, king-size stage and 'dial a light' facility—a three-way illumination system giving a choice of reflected light, transmitted light or a combination. The Stereozoom Series (5 and 7) with exclusive Power Pod gives real

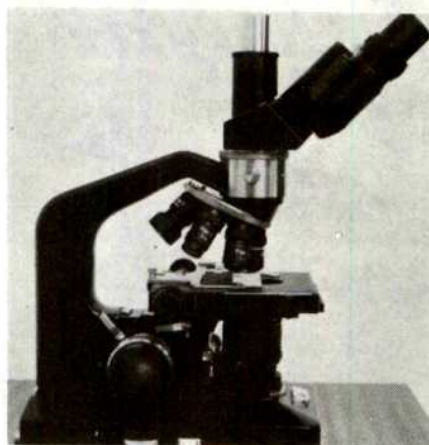


Bausch & Lomb Stereozoom

3-dimensional images by means of the Greenough design which features two complete converging optical systems that have a uniform optical path from eyepiece to objective. Both systems are aligned to focus at a single point assuring true 3-dimensional imagery, as each system fully utilises its own lenses. Zoom optics are standard and the sealed Power Pod prevents dust from entering the optical system, also stops image jump and image blackout. A flat-field optical system is integral and flexibility of attachment options covers a variety of Power Pods, eyepiece magnifications, supplementary lenses, stands, illuminators, polarising and photographic accessories.

Circle No. 52 on Reader Enquiry Card

The Tiyoda Super Widefield MT-B Microscope, available from **Hall Bros (Optical) Ltd** is a new concept in microscopy. This microscope is unique in design and construction, the designers having consulted professionals from all branches of medicine and science. Super Widefield eyepieces allow fatigue-free viewing of a field 90% larger than that seen with conventional widefield

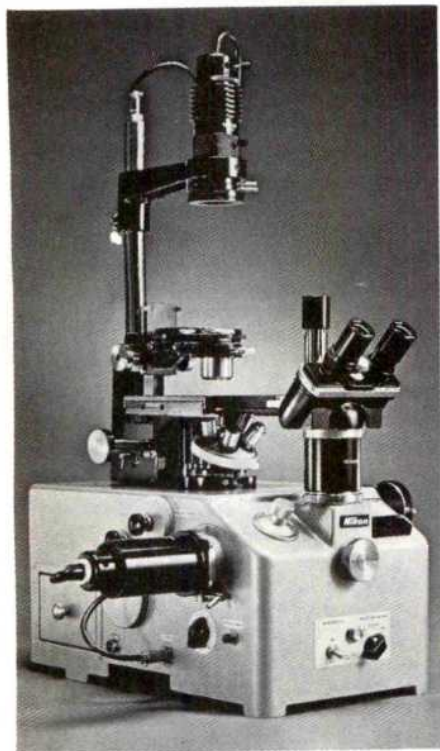


Hall Bros Tiyoda MT-B

eyepieces and a new patented 11 element Apochromatic condenser complements the Plan Apochromatic objectives $\times 2$, $\times 4$, $\times 10$, $\times 20$, $\times 40$ and $\times 60$. With these there is a $\times 1$ Plan Achromat which is perfectly parfocal with the other objectives. All controls are grouped together in one zone, letting the operator control the focus, aperture, stage movement and condenser adjustments with ease. For the first time super widefield photography is possible. A $\times 0.66$ lens is built into the phototube, and a low power photo-eyepiece combines to cover a rectangular format extending right to the edge of the visual field, e.g. with the Plan Achromat $\times 1$ objective an area of 14×22 mm can be recorded on 35 mm film.

Circle No. 53 on Reader Enquiry Card

Nikon manufacture a wide range of microscopes and ancillary equipment; research microscopes of various types—the Apophot, the Multiphot and the Biophot, conventional optical microscopes (the L-Ke, S-Ke and S-KT ranges), polarising (Model POH-3) and fluorescence (Model FL/FT) microscopes, stereoscopic (Model SM), metallurgical (Models ME, MSE, S-Epi, hole and surface-finish microscopes), inverted (Models M, MS and MTD), photo-ciné micrographic equipment and a wide range of interchangeable accessories. The Apophot has a sturdy console construction, a trinocular eyepiece tube, built-in magnification



Nikon Inverted Microscope

changer, interchangeable nosepiece system, a variety of stages, interchangeable condenser system, zoom Koehler illumination and various light sources available, Phase contrast (PC), interference-phase (IP), differential interference (DI) and polarising (P) attachments, also photomicrographic (PM) and ciné facilities and optional projection screen ($\times 6$ to $\times 3,000$). The Multiphot is capable of large-format photomacro- and photomicro-photography. The Biophot can be used for photomicrography, has a trinocular nosepiece tube, interchangeable arms, six-objective revolving nosepiece, filter facility, large stage and swing-out condenser, Koehler illumination, interchangeable eyepiece tubes and stages, P, IP, DI and PM facilities. The M, MS and MTD range of inverted microscopes, are particularly useful for tissue culture research. Interchangeable eyepiece tubes, PC, IP, DI and P facilities, also cine PM attachments, projection

screens and a specimen incubator. (Model M magn. $\times 6$ to $\times 2,000$). Model MS has a diascope illuminator and can be supplied in a biological or metallurgical form ($\times 30$ to $\times 2,250$; for PM, $\times 20$ to $\times 1,500$). Model MTD is specifically for tissue culture research ($\times 100$ to $\times 200$). Models ME, MSE, S-Epi (metallurgical) have magnifications in the range $\times 25$ to $\times 2,700$. The SM range of stereoscopic microscopes include the SM-5 ($\times 10$ to $\times 60$), the SMZ-2 stereoscopic zoom microscope ($\times 4$ to $\times 120$), the SMZ-6 ($\times 45$ to $\times 120$), various bases, pillars, stages, stands and PM facilities. The POH-3 polarising microscope has an extra long range of stage elevation, interchangeable single and multiple nosepieces, built-in transformer and Koehler illuminator, interchangeable eyepiece tubes, graduated, circular rotatable stage and epi-illuminator. Models FL/FT fluorescence microscopes have all types of filters, PC and PM facilities. **Circle No. 54 on Reader Enquiry Card**

Graticules Ltd have a comprehensive range of eyepiece and stage micrometers, counting and sizing graticules and the England Finder. Recently developed products include measuring instruments incorporating graticules, pocket measuring magnifiers and portable measuring microscopes. The Co-ordinate Measuring Microscope is a travelling microscope allowing measurements to be made over an area of $250\text{ mm} \times 250\text{ mm}$. Accessories include a rotary work table, light box, electronic digital readout, projector or 45° viewing heads and a choice of objectives, eyepieces and graticules.

The Portable Measuring Microscopes (Series 800) are versatile hand held microscopes calibrated to read down to

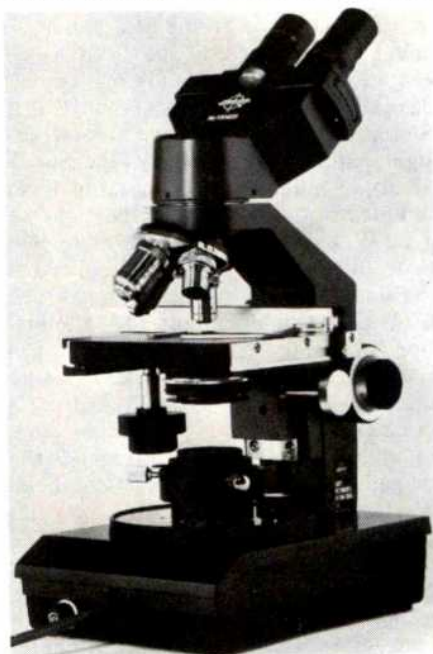


Graticules Series 800

$5\text{ }\mu\text{m}$. Accessories include miniature light fittings, spray droplet analysing graticules and plated through hole optics sets. The series 800 are designed for use on the work bench, in the laboratory, drawing office and so on, ideal anywhere where a full size microscope would be inconvenient to use and difficult to set up.

Circle No. 55 on Reader Enquiry Card

Pyser Ltd. for both teaching and research applications, the Swift M1000E series microscopes have a newly-designed base and a low-cost phase contrast unit. The base incorporates pre-centred 6 V 20 W built-in halogen illumination, the brightness of which can be adjusted by means of variable light control. The semi-Koehler illumination system is fitted with a centerable field iris; an alternative mains base with a built-in 240 V 30 W lamp can be supplied. Monocular, binocular,



Pyser Swift M1000 E

trinocular and tutor-tube versions are available; also a wide choice of stages to meet varying budget requirements. The plain stage is drilled to take stage clips or an attachable mechanical stage with controls on the top; a built-on mechanical stage has coaxial controls at the side while another built-on mechanical type has low-position vertical drive coaxial knobs. Coarse and fine focusing operates on the stage, which moves on precision ball bearings. The coarse adjustment is fitted with a patented clutch. These instruments can be used for dark and bright ground microscopy as well as phase contrast. In addition to the normal ranges of achromats and plan achromats, new objectives of $\times 40$ and $\times 100$ have been introduced for both bright field and phase contrast in the semi-plan form. **Circle No. 56 on Reader Enquiry Card**

nature

30 June 1977

Carry on talking

YOU MIGHT think that, what with the Select Committee on Science and Technology, the Parliamentary and Scientific Committee, The Royal Society, the Council of Engineering Institutions, the British Association for the Advancement of Science, the Council for Science and Society, Chief Scientists in governmental departments and an Advisory Board for the Research Councils, there is no shortage of channels in Britain by which scientists could make their views known to politicians and by which politicians could learn more about science and technology. You would be wrong. Scientists and politicians generally only talk to each other when things are going wrong, and even then it is usually directors of laboratories or scientific administrators who speak for science. A modest welcome, then, for the initiative of the Anglo-German Foundation for the Study of Industrial Society in trying a new format.

The foundation, in collaboration with Mr Ian Lloyd, MP, chairman of the Select Committee's sub-committee on science, recently invited a small number of MPs and staff members of the committee to spend a day and a half listening to six scientists and engineers describing their fields of interest, the prospects they see and the difficulties they face, not simply in the intellectual sphere but also in securing adequate support. The topics covered were construction,

power generation, immunology, genetics, microelectronics and the earth sciences.

In many ways the initiative was imaginative: the group was small enough for round-the-table conversation to flow easily, the scientists and engineers had ample time to describe their work, no one was angling for funds or speaking for the public record. On the other hand the MPs, for whom the show was staged, were conspicuous mostly by their absence; by half way through the gathering, Mr Lloyd was the only one left. There were, no doubt, other pressing matters to be attended to, and the harsh realities of the ballot box make little allowance for how well an MP has briefed him or herself, least of all in science or technology. So the welcome for the initiative must be tempered by some concern about whether politicians really value such exchanges. And if they do, the foundation has to decide on what lines another meeting should run. Should MPs be treated to completely new faces each time or should the effort be made, whatever the accusations of cosiness, to keep a nucleus of scientists and engineers together in the hope that good relations will develop? There is much to be said for the latter policy, tempered, of course, by new faces where necessary. For the initiative should develop into a talking shop, not a lecture series. □

Whose venture, whose gain?

THERE were signs at a recent meeting in Basel that the rising risk-consciousness reflected, for instance, in the endless debate on recombinant DNA and in the witch-hunt for environmental carcinogens, is increasingly losing the sympathy of research scientists. The meeting was not about potential environmental hazards from research; the scientists were all there at the invitation of Hoffman-La Roche to celebrate their research director's 60th birthday with a series of talks on the contribution of fundamental research to the medicines of tomorrow. Participants however seemed almost equally preoccupied with the possibility that the fruits of that research might never reach the market.

The main source of unease, of course, is the fear that far-reaching defensive action may be taken on the basis of questionable evidence. This has already happened with saccharin in the USA; whether in the end the saccharin link with bladder cancer is or is not substantiated, the fact remains that the evidence on which it has been condemned is widely regarded as inadequate. Professor Peter Cerutti implied that the over-enthusiastic use of the Ames test for carcinogens could very well lead to similar over-reactions. The Ames test depends on the correlations between mutagenic effects of chemicals on bacteria and their carcinogenic effects in whole animals. But the bacteria used in the tests are much more sensitive than animal cells, whose repair mechanisms for chromosomal damage give them complete protection from chemicals at doses that would produce a positive result in Ames bacteria.

As Professor Burgen unarguably remarked in his summing-up, "We must be cautious but not timid". After

all, nothing venture, nothing gain. But one does have to be sure that it is not the public health that is being ventured for the gain of pharmaceutical companies. And from that point of view, it is not at all clear that the public is its own best friend. A recent survey by Sir Richard Doll and his colleagues, for example, illustrates once again how enormously doctors over-prescribe anti-microbial psychotropic drugs (*British Medical Journal*, 1, 1561; 1977).

The hazards of over-use of antibiotics are well known, and the natural ability of virulent strains to acquire resistance to drugs has often been emphasised in the course of the recombinant DNA debate. The hazards of psychotropics, of which the most popular are Roche's own Librium and Valium, are not as well known. But as the authors of the *BMJ* article point out, with about 10% of males and 20% of females now taking psychotropic drugs, it is necessary to ask questions about, for instance, their possible effects on driving and other skills that require judgment.

This is not a case of the pharmaceutical industry foisting on the public drugs which carry unacceptable risks; antibiotics are life-savers, and psychotropics are certainly invaluable in some circumstances. It is the unnecessary use of the drugs that creates the unacceptable risk. However, people are not rational about the acceptability of risks. Recent publicity shows that they will insist on being allowed (or even encouraged) to smoke, or to drive without seat belts, both of which carry large known hazards, and to accept the risk of disease to an unvaccinated child rather than minuscule risk of damage by vaccination. □

Nutrition planning: the poverty of holism

Donald S. McLaren, of the Department of Physiology at Edinburgh University's Medical School, criticises the approach of the planners to the problems of world nutrition

SINCE the World Food Conference in Rome in 1974 there has been a rising tide of proposals for nutrition planning, policies and strategies on both national and international scales. The United Nations, for example, has given its blessing through the FAO/WHO report on 'Food and Nutrition Strategies in National Development', and has further encouraged the trend through the choice of 'The importance of national and international food and nutrition policies for health development' as the topic for technical discussion at the recent World Health Assembly. But the proposals are shibboleths of holism that, lamentably, have gone unchallenged.

The holistic approach has undoubtedly had its triumphs, where the problem was appropriate and the means available made it amenable. The Manhattan Project and the American and Russian space programmes are obvious examples. But the disappointing results in medicine of the American Heart, Stroke and Cancer programme and of Britain's National Health Service, and the failure of numerous attempts to control population increase, suggest that in certain areas of social action guerilla tactics and not a war machine are required, giving flexibility, accountability and proven effectiveness. Nutrition is just such an area.

The new breed of nutrition planners have a disturbing predilection for coining vague neotechnologisms and drawing maze-like flow (or are they ebb?) diagrams. That is bad enough. But they also choose to ignore those harsh realities that do not fit into their preconceived schemes. Ironically, a prime example is the phenomenon as ugly as its own 'neotechnological' name: Agripower. The probing of the United Nations Research Institute for Social Development and others, for example, recently highlighted by Susan George in *How the Other Half Dies: the Real Reasons for World Hunger*, show how the world's poor and hungry are increasingly at the mercy of vast agri-business corporations (chiefly from the United States), of Western governments with their food 'aid' policies, and of supposedly neutral multilateral development organisations, like the World Bank—all of them, so the argument runs, working in collusion with local elites nurtured and protected by the powerful in the developed world.

Then there is the case of *Scientific American* devoting an entire issue to food and agriculture. The problems of food and nutrition were there dismissed as being entirely technological in nature and capable of solution by "additional technology and capital from the developed countries"—a root cause rather than a solution. Ironically, the numerous lavish advertisements documented the frightening story of US agripower that was shunned in the text. Rarely do the institutions responsible permit themselves to make statements on the ethical implications of their activities. It probably takes someone like the former US Secretary of Agriculture, Earl Butz, to admit that "Food is a weapon. It is now one of the principle tools in our negotiating kit"—and we know what happened to him.

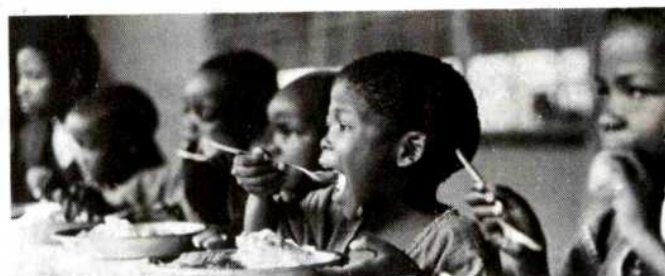
What has been ignored throughout such deliberations is the different nature of the two problems of food supply and human nutrition. It was this misconception which contributed to the failure of the UN Freedom from Hunger Campaign in the mid-1960s and to the Great Protein Fiasco of the early 1970s, as I pointed out in *The Lancet* at these times. Food shortage is a necessary but not adequate cause of undernutrition. There is many a slip betwixt the cup of

food supply and the lip of hungry man. Problems with the former are necessary, but not adequate, causes of those of the latter. That is why the Green Revolution has not resulted in less malnutrition.

Nutrition planners state that past efforts failed, if failed they did, because they were piecemeal and for no other reason; they claim that a new planned approach must succeed because it is holistic and not piecemeal. Now the term 'whole' has two senses, as Popper indicated in *The Poverty of Historicism*: the totality of all the properties or aspects of a thing; and the special properties of certain things which make them appear to be an organised structure rather than a 'mere heap'. Popper's argument is clear: though wholes in the latter sense have often been made the object of scientific study (notably by the 'Gestalt' school of psychology), in a social context the 'organised structure' in no way applies and holism is inappropriate. Methodological problems have not been surmounted and there is no reliable procedure for building hypotheses based on well devised observations, experiments or rational deductions. Holistic or Utopian social engineering is always public and not private, aims at remodelling the whole of society and extending the power of the state, and smacks of tyranny. The alternative is piecemeal social engineering, the word piecemeal having no pejorative sense but meaning just what it says—piece by piece or part at a time.

Past achievements of the piecemeal approach to nutritional problems, it is true, are not impressive. Iodisation of table salt is effective against simple goitre. Fortification of staple foodstuffs has helped to maintain optimum micronutrient status in many populations. Prevention of the most common deficiency, iron, remains unachieved everywhere. Classical vitamin deficiencies have been influenced most by political action and social change—the disappearance of beriberi and xerophthalmia from Japan in the early part of this century, of pellagra from the southern United States initiated by the immigration laws in the 1920s, and the emergence of rickets in British Asian immigrants. The widespread intractable problem of the malnutrition-infection complex of early childhood in developing countries has deep socio-economic roots. Mothercraft and nutritional rehabilitation centres have made modest but real contributions.

In those communities where overnutrition and nutrition-related degenerative diseases are rife, it remains to be seen whether the health of the increasingly diet-conscious public will benefit from the multifarious and often conflicting advice it is being offered. In the meantime the fact is that nutrition remains largely one of Medawar's 'kitchen arts'—a science insufficiently underpinned by theory. A halt should be called to the present mania for holistic day dreaming. Research efforts should be renewed to bring order to the present 'mere heap' so that sounder measures may result, piecemeal though they may be. The WHO's resolution at its recent assembly, urging the eradication of the severe clinical forms of malnutrition by the turn of the century, offers at least some hope. □



Hospital meals: effective against malnutrition?

To get more than enough

Colin Norman reports on a massive research effort to combat hunger and malnutrition proposed in Washington last week

A RECORD harvest in the key grain producing countries last year and the prospect of another bumper wheat crop in the United States this year have temporarily removed famine and hunger from the daily headlines. But, with international grain reserves still at a dangerously low level, a catastrophic food shortage in many parts of the world is still only one poor harvest away. Moreover, even if luck continues to hold, there's little room for complacency: according to widely accepted estimates, between 500 and 1,000 million people are already chronically underfed, and rapidly increasing population in most developing countries is continuing to drive up food demand.

Against that grim background, it may seem like the height of folly to suggest that "it should be possible to overcome the worst aspects of widespread hunger and malnutrition within one generation". But that is the conclusion of a major study published last week by the National Academy of Sciences*. One of the most intensive investigations of world food prospects yet put together, the study took two years and involved some 1,500 people; the effort was coordinated by a committee headed by Harrison Brown, professor of geochemistry at Caltech.

The committee's optimism is founded on the expectation that a massive research effort on a broad spectrum of agricultural, social and population problems could lead to a doubling of agricultural production in the developing countries and more equitable distribution of food during the next quarter century. Such an expansion would, in theory, be sufficient to cope with increased food demand from rising population and affluence, and it would also provide an increase in food consumption per capita worldwide.

But the committee is also quick to acknowledge that science and technology will not alleviate widespread hunger by themselves. Basic social changes will be needed to ensure more effective distribution of available food and to reduce poverty, though the committee's report does not advocate any specific social remedies. More attention to nutrition policies will also be required in both the poor and affluent countries, and there must also be renewed efforts to hold down the explosive rate of population

growth, the committee states.

Given the complexity of the world food problem, the committee's optimism is surprising. During the past few years there has been a fundamental shift in the food picture as grain reserves have dwindled to less than a third of their 1970 level and more countries have become dependent on the North American breadbasket to meet their food demands. After a decade of stability, crop prices have fluctuated wildly, aggravating problems of hunger and malnutrition in the poorer countries. And the increasing reliance on North American agriculture to meet world demands raises the grim possibility that a failure of the United States and Canadian harvest would have dire consequences.

Numerous and complex

The roots of the problem are numerous and complex, but one of the chief factors has been rising population in many developing countries which has caused food demand to outstrip production. According to figures in the Academy's report, although food production in the developing countries rose at an unprecedented rate of 2.8% per year between 1960 and 1975, demand for food increased by 3.5% per year. Consequently, a growing number of countries have joined the long list of food importers and there has been virtually no increase in per capita food consumption in the developing countries.

And the prospects for population growth don't give much room for optimism. Although there are recent signs that world population growth is beginning to abate, there are likely to be at least 6,000 million mouths to feed by the year 2000, and some 90% of the growth will take place in the developing countries. World population has recently passed the 4,000 million mark. To feed that many people and provide even a modest increase in per capita consumption will require Herculean efforts. According to the Academy study, the developing countries will need to increase their food production by about 3-4% per year, while stepping up efforts to reduce their birth rates and providing a more equitable distribution of food.

The task will require a major infusion of funds into research, particularly in the United States, and structural reforms in research programmes and in the political and agricultural systems of many countries.



High yield rice under test

Though the committee accepts that the task will not be easy, it states that "a latent political will now exists in numerous countries which could be mobilised in a mutually supporting fashion to commence and support such efforts".

In the past, increases in agricultural production have largely been won in two ways: increasing the acreage under cultivation and improving yields per acre. There have recently been sombre warnings, however, that marginal lands are being taken out of production in some parts of the world because of such problems as desertification and soil erosion and flooding caused by deforestation. The prospects for increasing agricultural acreage are therefore poor, and the Academy study predicts that annual increases in crop area will be unlikely to exceed 1% per year over the next 25 years. The bulk of the increase in agricultural production will have to come from boosting yields per acre.

The committee offers a shopping list of research programmes which it says should be stepped up to help increase yields. They include the development of fertilisers for use on tropical soils, efforts to improve irrigation and soil conservation, methods of reducing crop losses between the farm gate and the market, the development of more efficient farm systems and the application of biological methods of pest control. Two programmes are expected to be crucial to the long-term prospects for increasing yields, however. They involve boosting the efficiency with which crop plants use solar energy through photosynthesis, and efforts to develop cereal plants capable of utilising nitrogen directly from the atmosphere instead of having it supplied as chemical fertiliser.

At present, some 45 million tons of fertiliser is used per year worldwide. To bring about a doubling in food output during the next quarter century

*World Food and Nutrition Study, Printing and Publishing Office, National Academy of Sciences, 2101 Constitution Ave NW, Washington DC 20418, \$6.95.



A must to avoid

would require between 2 and 4 times that amount, the Academy committee reckons. That would require about 500 new fertiliser plants, at a cost of some \$50,000 million at today's prices. Such a vast use of fertilisers would not only be inordinately expensive, but it would also lead to environmental problems through run-off into streams and rivers. Hence the importance of efforts to produce crop plants which can fix their own nitrogen.

It has long been known that leguminous plants have a symbiotic relationship with bacteria living in their root systems which fix nitrogen from the atmosphere and supply it to the plant in a usable form. The immediate goal of research efforts is to improve that symbiotic relationship, thereby increasing yields of leguminous crops without increasing the application of fertilisers. In the more distant future, research efforts are aimed at inducing similar symbiotic associations between bacteria and cereal plants and, perhaps, transferring the genetic capability to fix nitrogen directly into the cells of crop plants.

Each of those goals requires fundamental biological breakthroughs, however, and they may also involve ecological problems. Though researchers are optimistic, there's no guarantee that the required breakthroughs will be forthcoming. The Academy committee recommends a major boost in funding.

The second key effort, boosting photosynthetic efficiency, also requires substantial breakthroughs. At present, most crop plants capture only about 1-3% of the solar energy they receive, but some plants, such as corn and sugarcane, do much better. Such plants seem to possess a natural mechanism for suppressing the light-aided consumption of stored foodstuffs, and the hope is that research into the underlying processes will lead to methods of increasing the photosynthetic efficiency

of other crop plants. The potential is vast: the Academy report reckons that yields of some crops could be boosted by between 50 and 100% if the research produces results. The report therefore recommends a substantial boost in funds for such research.

Although those two efforts undoubtedly provide the major hopes for a technological fix for increasing food supplies, they are both long-term, long-shot efforts. Less glamorous projects, such as improved plant breeding, soil management and pest control are likely to be more certain and yield quicker results: they should thus be pursued equally vigorously, the committee states.

Increased social research

In the long run, social and organisational changes are likely to prove just as important as technological innovations in alleviating world hunger, and thus the committee urges a sharp increase in social research. In particular, noting that countries with such diverse social and political systems as Taiwan, the People's Republic of China and Sri Lanka have managed to increase food production while lowering birth and death rates, the committee recommends a programme of comparative analysis to see whether any generalisations emerge. "Social science research on food and nutrition lags significantly behind that of biological research because of shortages of skilled personnel, suitable methods of research, data, prior research findings, research funds and effective organisation", the committee states.

Ultimately, political and social changes to combat poverty are likely to be the most effective means to reduce hunger. They are, however, less easy to achieve than changes in the technology of food production. The Academy is nevertheless relatively sanguine about the prospects. □

The Academy committee's report calls for a sharp increase in agricultural research on food production and social research on food and nutrition policies. A large proportion of the effort will probably have to be undertaken in the affluent countries, particularly in the United States, but the committee urges that steps be taken to build up the research capacities of the developing countries, and that the international network of research centres be strengthened and expanded. The chief recommendations:

- Research spending on food and nutrition problems should be increased by \$210-230 million a year in the United States. A large share of the increase should go to the Department of Agriculture, whose research budget is now about \$700 million a year. The National Science Foundation, the National Institutes of Health and the Agency for International Development should also be given large increases in their research support.

- A new programme of competitive research grants should be established in the Department of Agriculture for work on basic problems such as increasing photosynthetic efficiency and biological nitrogen fixation. (The Ford Administration in fact proposed that \$28 million be spent on such a programme in fiscal year 1978, but the House Appropriations Committee recently reduced the amount to \$10 million.)

- A new post should be established in the Department of Agriculture with responsibility for research and education.

- The United States should train more researchers from developing countries and provide more direct support for establishing facilities and research institutions in developing countries.

- Two new responsibilities should be added to the Executive Office of the President: an office to develop and maintain a coherent strategy for dealing with world food and nutrition problems, and another to coordinate US and international efforts (in fact, such tasks will probably be given to the Office of Science and Technology Policy.)

The committee briefed White House officials on the recommendations last week and, though the reception was said to be sympathetic, the discussion concerned bureaucratic problems in the Department of Agriculture rather than research strategies.



Harrison Brown, committee coordinator

Towards a pollution policy

Richard Sandbrook outlines how a UNEP plan could help to save the Mediterranean from pollution

THERE are many indicators that the so-called environmental debate has moved on from its more sensational phase into a new and more difficult stage of human endeavour, at least as far as pollution questions are concerned. The catch phrases—'limits to growth', 'spaceship Earth', 'the environment revolution'—do not excite the media or its readers any more, much less governments. Anti-pollution legislation is no longer pushed through the world's parliaments, the UN or the EEC on a wave of public alarm and high idealism. It is becoming increasingly necessary to present solid scientific evidence of harm and an economic evaluation of benefits and costs before controls gain political support.

This trend is becoming evident in certain sections of the United Nations Environment Programme (UNEP) too. Born in Stockholm in 1972 on a crescendo of 'environmental' enthusiasm, it is now struggling to survive in the harsh world of international law, inflation-prone economies and hard scientific evaluation. But in one area at least UNEP has made this shift successfully. Its Mediterranean Programme is one of the most complex international pollution control and environmental protection exercises ever undertaken. It reinforces the trend toward regional rather than global approaches to environmental protection—at least for the marine environment; and it helps to marshal together many of the previously competing UN agencies, governmental institutions and independent research units that can play a role.

Intergovernmental consultations on the programme started in February 1974, with the Food and Agricultural Organisation (FAO) meeting on the protection of living resources from pollution. UNEP became involved that year and in February 1975 convened an intergovernmental meeting in Barcelona on the general protection of the Mediterranean. The gathering discussed the first draft of a framework or 'umbrella' convention for the protection of the Mediterranean, and approved a three-part programme encompassing legal arrangements, scientific research and monitoring and integrated planning for the region.

A year later, in February 1976, the umbrella convention was finally approved by 16 of the 18 Mediterranean

states (the exceptions being Algeria and Albania), together with two specific protocols attached to it on the prevention of pollution by dumping (ships and aircraft) and on intergovernmental co-operation in the event of a pollution emergency. The convention was very broad, committing states to "take all appropriate measures . . . to prevent, abate and combat pollution of the Mediterranean Sea area and to protect and enhance the marine environment". A signed convention is far from a ratified convention, however, and a ratified convention is in turn far from a working one. The 1969 amendments to the IMCO 1954 oil pollution convention, for example, only came into force after the required number of ratifications in January of this year; it will be a further year at least before it is working.

Will the Mediterranean agreements suffer the same fate? One suspects not, for the convention and the two protocols to date are but a part of the much wider environmental programme—driven on, in the northern Mediterranean at least, by the threat of reduced tourism, waterborne health risks and highly contaminated marine life locally. In March 1975, for example, the French Institute of Medical Oceanography issued a warning that if mercury pollution continued at its current rate then fishermen and others who consumed on average two kilos of certain local fish a week would be chronically ill within 7 years and dead in 20 from mercury poisoning.

The legal arrangements, for example, are not going to end with ratification of the three existing instruments. A further protocol on land source pollution could be ready by the end of 1977 to be added to the framework convention, and a further protocol on exploration and exploitation of the seabed and the continental shelf is planned.

Questioning the rationale

The rationale for this grand plan, at least on an international level, is often questioned. Why not leave pollution control up to individual states or at most neighbouring states? Is it simply following a fashion set by the Oslo and London dumping conventions and the Paris convention on land source pollution? Or do the coastal states really believe that an integrated and regional approach is necessary?

The geography of the region provides some explanation. The Mediter-

anean Sea is a semi-enclosed sea and its water is effectively divided into two basins (north and south) which rotate cyclonically. In this way pollutants cross national frontiers; those from Italy potentially affect France, and those from the Nile Israel. The currents hug the coasts and such ship-source pollutants as floating litter and tar tend to be spun centrifugally toward them. Saline water enters the sea in the surface waters of the Straits of Gibraltar and leaves by the same route at greater depth. The average estimated residence time of the water is of the order of 80 years, although the range is probably from a few to several hundred years. This in effect means that for those long-lived and stable pollutants accumulation over time is bound to occur.

Secondary to the transfrontier rationale is the monitoring and research needed to underpin environmental controls. Given that the first step in controlling any pollutant is to know its source, its concentration and then its effect, the idea of monitoring the sea as a whole for the three sets of variables makes eminent good sense politically and for the more mobile and persistent pollutants good sense scientifically. This monitoring work is now progressing on seven fronts throughout the Mediterranean basin:

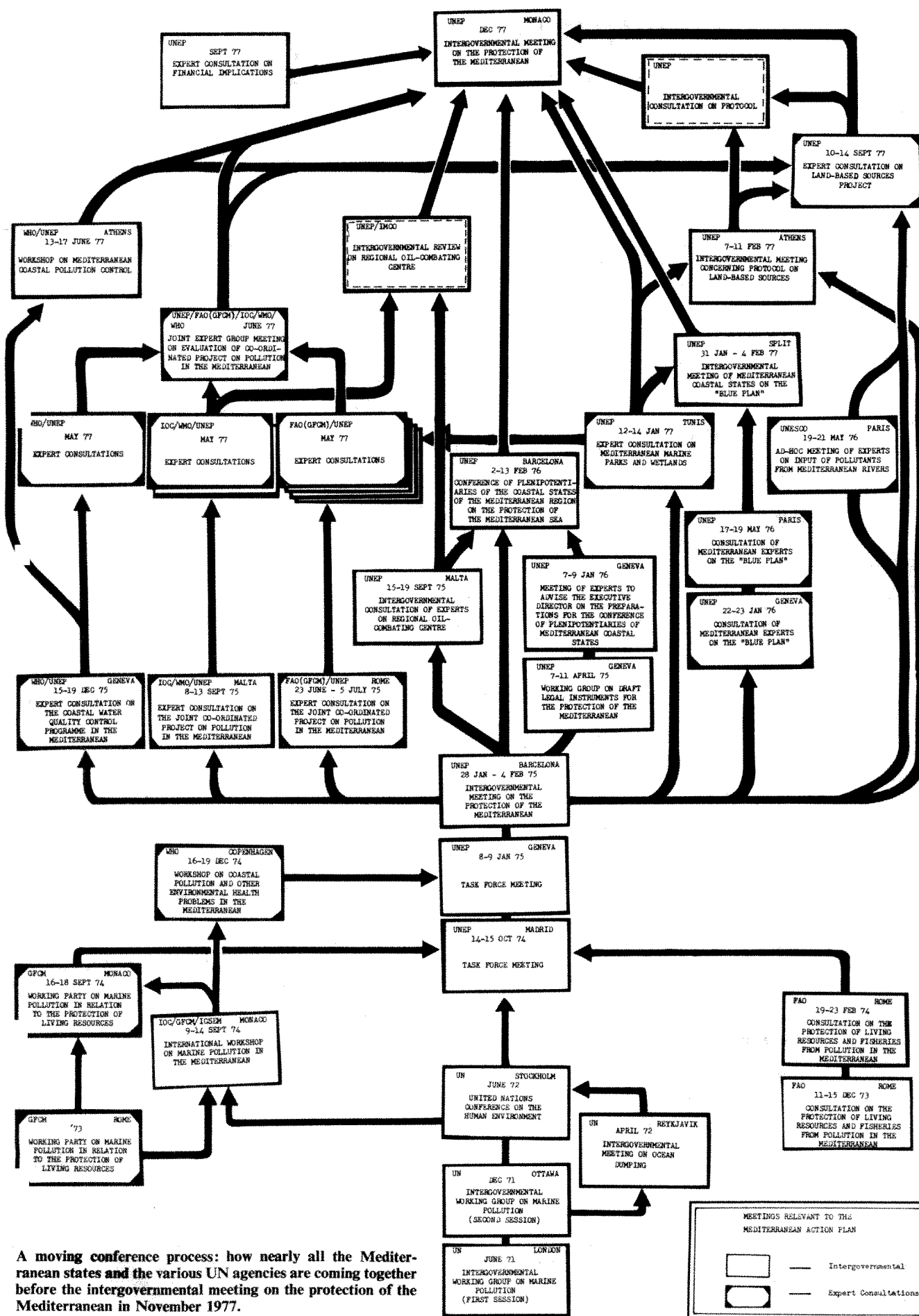
- Baseline studies and monitoring of oil and petroleum hydrocarbons, in conjunction with the Intergovernmental Oceanographic Commission of UNESCO and the World Meteorological Organisation. This programme, which now involves 11 of the 16 coastal states and 26 national laboratories, is focused on the new regional oil combating centre in Malta, which is run by IMCO.

- Baseline studies and monitoring of the heavy metals, particularly mercury and cadmium in marine organisms—a project sponsored by FAO and UNEP in 14 countries and 38 laboratories.

- Baseline studies and monitoring of DDT, PCBs and other chlorinated hydrocarbons—an FAO/UNEP project in 13 countries.

- Research on the effects of pollutants on marine organisms and their populations (FAO/UNEP in 11 countries). The aim is to study the effect of various pollutants on the population dynamics of certain test organisms; it is hoped the study will lead to the identification of particularly vulnerable species

*The 1972 Oslo Convention for the Prevention of Marine Pollution by Dumping from Ships and Aircraft entered into force in 1974 and covers the North Sea and the northwest Atlantic. The London Convention is a global convention to control the dumping of wastes at sea, and entered into force in 1975; it is now administered by IMCO. The Paris Convention for the Prevention of Marine Pollution from Land-based Sources covers the same area as the Oslo Convention and is not yet in force.



A moving conference process: how nearly all the Mediterranean states and the various UN agencies are coming together before the intergovernmental meeting on the protection of the Mediterranean in November 1977.

within the different tropic levels at various stages of their life cycles and in time lead to the identification of indicator species for water quality criteria.

- Research on the effects of pollutants on marine communities and ecosystems (FAO/UNEP in 12 countries). The project will amount to the intensive field study of various coastal ecosystems under stress, or of areas where ecosystem changes may be anticipated as a consequence of development and areas that are unpolluted and designated as marine parks for control purposes.

- Problems of coastal transport of pollutants (IOC/UNEP in 12 countries). The aim is to study the water circulation in coastal areas to understand better the physical transport of pollutants.

- Coastal water quality control (WHO/UNEP in 8 countries). The overall objective of this pilot project is to produce statistically significant data on pollution levels that may affect human health.

Each of these seven projects involves a nominated 'activity centre'; also needed is the back-up support of the IAEA and UNEP on intercalibration of equipment and analytical techniques and the provision of common maintenance services for the more sophisticated laboratory instruments. A technical training programme is under way; grants for instruments and a growing number of manuals and guidelines for the participating laboratories are available.

All of this will be the backdrop against which the next protocol will emerge. It will take a form broadly similar to that adopted in Paris in 1974 for the North East Atlantic, in Helsinki in 1974 for the Baltic Sea Area and in Brussels in 1976 for European Economic Community waters. As it is being drafted midway in the seven point scientific programme, however, the hope is that after a series of evaluation meetings of the projects involved, it will avoid some of the pitfalls that the precedents have fallen into. For example, the interim body set up to help the process of ratification of the Paris convention (IPARCOM) is the arena for much disagreement. Typical questions to be answered are: What constitutes pollution? What should be monitored? And by whom and where? For the convention simply states that by pollution is meant substances or energy introduced directly or indirectly by man that results in such deleterious effects as hazards to human health, harm to living resources and marine ecosystems, damage to amenities or interference with other legitimate uses of the sea. What is meant by 'hazards', 'harm' and 'interference' is unclear.

And on top of the work of IPARCOM (and any Mediterranean protocol developed) are the problems imposed by the European Commission directive on land source pollution (1976).

Black and grey lists

Both the Paris convention, and its later brother, the Brussels directive, include two lists (as annexes) of substances to be considered. The so-called black lists in the Paris convention relate to substances to be eliminated from the environment—for example, mercury and its compounds, persistent oils and hydrocarbons of petroleum origin, cadmium and cadmium compounds—and in the Brussels directive a similar list which is to be subject to uniform emission standards with an option for states to operate as-yet-undefined "environmental quality objectives". In both there are grey lists of substances that will be subject to less strict controls.

The treaty language is always, of course, strong enough to sound impressive environmentally—even, as some would allege, economically. One admitted purpose behind the Brussels approach is that all states should suffer the same environmental constraints no matter where the pollution occurs—an 'equalising' of competition. But in practice things are far from simple. There is little or no quantitative evaluation of acute or chronic harm to back any standards that may be set, and no recognition of the fact that receiving environments differ. For those who want to delay ratification this provides excuse enough. But a less cynical view is that unnecessary controls are not in anyone's interests other than the pollution control equipment manufacturer—and so why have them? The British have become so upset by the arguments over Paris and the Brussels directives that they have now resorted to publishing a detailed explanation of their case (DoE Pollution Paper No 11).

This time round, Mediterranean governments who have been involved in the EEC and IPARCOM process should have an idea of the likely costs involved, the necessary levels of control that should be applied and most particularly be able to avoid the problems of definition that bedevil the precedents in the field. Not least, by having some research first, many more governments will be aware of just what is under consideration.

The final concern of the programme is that of integrated planning; here the so-called Blue Plan and the Priority Action Programme are the two components. Throughout, UNEP has been at pains to stress that it is not posturing as an executive agency for the area—a sort of unofficial Brussels for the Mediterranean basin—and the Blue

Plan reflects this. There are no 'line' responsibilities envisaged but a set of 'processes' to be developed to enable governments to take the environment into account when making their own decisions. Thus systematic surveys of all major economic growth areas are called for, and so are studies of such varied topics as soil protection, water resources and urbanisation trends. The Priority Action Programme is in essence a part of the same plan but it includes areas ready for immediate action before the overall process gets underway. Again a series of cooperating national and international agencies are included.

The catch, of course, is how much notice governments will take of the environmental advice that they receive, and to what extent the whole effort will become bogged down in regional economic disparities. It all looks dangerously like the European Commission but without the teeth of its Council. To a certain extent, governments will become locked into a process that will ensure compliance—the major aid agencies (UNDP and the World Bank) that are helping some of the southern coastal states are going to be influenced by the development criteria agreed under the plan's auspices. Similarly, FAO who are developing the fisheries and WHO who are helping to build up sanitation infrastructures in the southern states are going to be closely involved. However, both components are small in resources. The first phase, costing an estimated \$1.5 million in total will, one suspects, be little more than an exchange between planners and development strategists.

This is not to knock too much. It is after all remarkable that Greece should sit with Turkey—Israel with Egypt—and talk over such important domestic strategies at all, and if the approach of the Mediterranean programme goes well it is likely to spread to other regional waters—the Persian/Arabian Gulf, the Caribbean Sea and the West African coastline for example.

There is no doubt that the unified scientific monitoring will lead to progress first in our understanding of the marine ecosystem and maybe then in controlling the obvious pollution that is going on. Governments are discovering disturbing levels of heavy metal contamination and waterborne disease in their waters. These facts are going to be made public one day and that is going to ensure a change in the level of investment and control exercised. Cousteau, who in 1972 said that the Mediterranean would surely die, admits that much progress. In 1976, he sent a telegram to UNEP saying that it might live after all. □

GENETICS

Full circle

The debate over potential hazards associated with recombinant DNA experiments has now turned full circle in the United States, with many of the people who first raised the issue in public arguing that legislation is unwarranted. Colin Norman reports

EXACTLY four years ago, a group of scientists attending the 1973 Gordon Conference on nucleic acids began to worry for the first time about the possibility that transplanting genes from disparate species into living viruses or bacteria might produce an organism with potentially hazardous properties. Discussions at that meeting led to a resolution calling for an investigation of such concerns, and the recombinant DNA debate has been picking up momentum ever since. Last week, at the Gordon Conference, some of the same scientists expressed alarm at where the debate has led.

An open letter, signed by 137 scientists at the conference—more than 75% of the attendees—warns that legislation now pending before Congress and before some state and local authorities in the United States could cripple recombinant DNA research. The letter claims, moreover, that the impetus behind the legislation comes from “exaggeration of the hypothetical hazards of recombinant DNA research that go far beyond any reasoned assessment”.

The Gordon Conferences are attended by scientists who are actively engaged in research on nucleic acids, and they thus represent people who would be directly affected by legislation. Their concerns are therefore not entirely devoid of self-interest, but they nevertheless probably reflect the views of a large proportion of the scientific community.

The call for a halt to legislation is, however, too late to have much effect and it could even be counter-productive. Committees in both the House and the Senate have now approved versions of legislation to regulate recombinant DNA research in the United States, and there is now strong and unstoppable momentum behind the legislative process. Moreover, there is a compelling argument for legislation: guidelines now governing federally-funded recombinant DNA research, issued last year by the National Institutes of Health, are not legally enforceable and do not formally apply to research supported by non-government sources.

The chief concern which led to the

formulation of the open letter at the Gordon Conference is that the legislation is likely to go well beyond simply putting the NIH guidelines into legal regulations. A bill approved by the Senate committee on human resources would establish an 11-member Presidential commission to regulate the research, for example, while a bill approved by the House health subcommittee would set up a complex procedure for approving some types of experiments on a case-by-case basis. Both versions call for new regulations to be drafted, and both would also impose stiff penalties on people caught flouting the rules.

The open letter claims that “the experience of the last four years has not given any indication of actual hazard”, and a number of scientists have recently testified before Congress that research indicates that the hazards may be much more remote than first thought. Such claims rest on evidence that there may be greater natural genetic exchange between species than once believed, that genes from higher organisms have not so far been found to be faithfully expressed in bacteria, and that bacteria bearing transplanted genes seem to be less capable of surviving in the environment than unmodified bacteria. The authors of the letter therefore suggest that while the hazards seem to be growing less plausible, controls on the research are getting more strict.

Nevertheless, few scientists are willing to argue that the research is entirely devoid of risk and thus should not be controlled. Moreover, opponents of the research have also

pointed out in Congressional testimony that evidence suggesting that the risks are less plausible is very preliminary, and that there is no justification for lessening the controls. Faced with those circumstances, Congress is not likely to drop its moves towards legislation because those most affected believe that strict legal regulations are unjustified.

The authors of the letter in fact tacitly concede that Congress is bent on legislation, and they thus urge that “should legislation . . . be deemed necessary, it ought to prescribe uniform standards throughout the country”. The idea is that Congress should prohibit state and local governments from setting their own controls on recombinant DNA research, but Congress is again unlikely to accede completely to the request. Both versions of legislation now under consideration would allow local authorities to set regulations which are at least as strict as the federal controls, but the local authorities would first have to prove that their additional restrictions are required to protect public health or the environment.

A few months ago, a number of prominent scientists reluctantly agreed that legislation is needed to ensure that controls on recombinant DNA research are applied equally to experiments funded by government or private funds. They also supported legislation on the grounds that it would stop local governments from setting their own controls. “Scientists were once urging Congress to pass legislation, and now they are telling us that legislation is unnecessary”, complained one Senate staff member last week. “That is not likely to help their case”. □

Open letter

The open letter signed by 137 scientists at the Gordon Conference reads:

“We are concerned that the benefits of recombinant DNA research will be denied to society by unnecessarily restrictive legislation.

“Four years ago, the members of the 1973 Gordon Conference on Nucleic Acids were the first to draw public attention to possible hazards of recombinant DNA research. The discussions which started at that meeting resulted in the issuance in 1976 of the NIH Guidelines for the conduct of this research.

“We, members of the 1977 Gordon Research Conference on Nucleic Acids, are now concerned that legislative measures now under consideration by Congressional, state and local authorities will set up additional regulatory machinery so unwieldy and unpredictable as to inhibit severely the further development of this field of research.

We feel that much of the stimulus for this legislative activity derives from exaggerations of the hypothetical hazards of recombinant DNA research that go far beyond any reasoned assessment.

“This meeting made apparent the dramatic emergence of new fundamental knowledge as a result of application of recombinant DNA methods. On the other hand, the experience of the last four years has not given any indication of actual hazard. Under these circumstances, an unprecedented introduction of prior restraints on scientific inquiry seems unwarranted.

“We urge that Congress consider these views. Should legislation nevertheless be deemed necessary, it ought to prescribe uniform standards throughout the country and be carefully framed so as not to impede scientific progress.

“The 137 undersigned are members of the 1977 Nucleic Acids Gordon Conference”.

SEVESO

Roche's reply

The debate continues on the Séveso disaster. Alastair Hay reports

In an effort to counter the criticism of its subsidiary Givaudan, the Swiss multinational pharmaceutical company Hoffman-La Roche has recently given wide publicity to an internal newsletter on the environmental hazards at the Italian town of Séveso. The town was contaminated on 10 July last year by an explosive discharge from a chemical reactor manufacturing trichlorophenol. The discharge contained an extremely toxic contaminant, dioxin.

Over the past few months many press, radio and television reports have referred to the deteriorating position at Séveso. These reports quite naturally directed attention to Givaudan, the owners of the Icmesa chemical plant responsible for the contamination problem, and have been extremely critical of the company. The Roche newsletter represents an attempt on the behalf of its subsidiary to redress the balance. It claims to produce as objective an account as possible of the picture at Séveso, speaking of "considerable progress" made in the decontamination effort and the settlement of private compensation claims, and it refers to the "encouraging" health situation.

But the newsletter is primarily concerned with the prevailing position, referring only in passing to the circumstances of the actual 10 July accident. The reactor's explosive discharge is referred to as a toxic mixture of chemicals which escaped from the plant "as a result of a runaway reaction". As the cause of the accident is the subject of a legal inquiry by an Italian court, Givaudan obviously prefers not to make public pronouncements about the case.

In private, however, a company spokesman maintains that even after months of tests they still do not know what caused the accident. Givaudan can hardly deny that its Icmesa plant was the cause of the problem, but the company denies vehemently the suggestion that it was in any way negligent. "Human error" is mooted as the probable cause of the explosion.

Company officials insist that Givaudan knew of the previous accidents in trichlorophenol plants—at least nine have been publicly documented since 1949, and it is thought there may have been as many as 14. The officials say Givaudan accordingly designed the plant to incorporate safety

features to protect employees. This meant that the safety valve and vent pipe were placed to discharge the reactor contents away from the confines of the factory—with the disastrous consequences that the July accident demonstrated. The company now admits that there may have been a "lack of foresight" in anticipating this event.

In response to accusations that the company withheld information on its reaction processes, Givaudan insists that it had all the necessary permits authorising operations on the site. As for the trichlorophenol reaction, dioxin production was constantly monitored and ranged from 10–30 ppb, well below the level recorded in other plants producing trichlorophenol destined for the manufacture of the herbicide 2,4,5-T.

The company maintains that two weeks was necessary to assess the extent of the contamination in the Icmesa neighbourhood after the accident, but that as soon as reliable data was to hand, it was passed to the Italian authorities.

One of the principal problems facing the Italian authorities and Roche was the paucity of clinical information on dioxin toxicity in humans. Dr Giuseppe Reggiani, director of clinical research for Roche, says the health situation at Séveso is "reassuring". He insists, however, that certain categories of high risk groups, in particular the children with chloracne and the residents of the heavily contaminated Zone A, be constantly monitored as part of an epidemiological survey.

The Lombardy regional authority is to undertake such a survey under the auspices of its Health and Epidemiology Commission. The commission proposes to measure some 22 biochemical parameters in a population they consider likely to have been in contact with dioxin. The authority expects Givaudan to pay for the operation. The company, on the other hand, insists that before government claims can be met, due weight will have to be accorded to one of their decontamination proposals, made in August of last year. This proposal was not accepted by the Italian authorities, but would have involved the spraying of vegetation with an olive oil-cyclohexanone mixture, which would have facilitated the photoreduction of dioxin present. Givaudan claims that its programme could have resulted in the destruction of 80–90% of the dioxin.

BRITAIN

Down wind

WITH the publication last week of a 70-page report on wind energy*, the UK Department of Energy (DEN) has now completed its first round of studies on alternative energy sources. Responding to a question at a press conference last week to introduce the report, the department's chief scientist, Dr Walter Marshall, said it was "unambiguously clear" that the source which was "top of the pops" was wave power. That was followed by solar power, then geothermal and tidal power.

Final judgment, according to the head of the DEN's Energy Technology Support Unit at Harwell, Dr K. Dawson, would depend on economic and environmental considerations as well as potential contributions to total energy needs. But it was clear, he said, that wind wouldn't solve the country's energy problems. Marshall himself accorded it low priority because, he said, he has doubts whether it will be environmentally satisfactory.

That small dampener does not mean the DEN is not going to pursue the matter. The department and an industrial consortium are each pitching £75,000 into what is called a "detailed design study for a large machine installed on a prime site"; another £10,000 is coming from two interested Scottish utilities. The idea is to obtain a firmer estimate of costs, to improve the confidence with which the current cautiously optimistic estimates are held.

But, as Marshall sees it, there is a problem. If it is environmentally acceptable to use the 3,000-odd identified sites and it is possible to put aerogenerators on them, the costs might be reasonable and wind energy might seem economic even now at current prices. But, he goes on, if there is less confidence about the sites and it is left to the market to introduce wind power, the number of sites used would be small, costs would be high and wind power would hardly be economic.

Last week's report was completed before Sir Martin Ryle, the Astronomer Royal, detailed his arguments favouring wind power in an article in *Nature* on the economics of alternative energy sources. Asked whether Ryle's arguments would be incorporated into DEN work, Marshall said that, now the DEN had finished its own calculations, he was trying to get people together, including Ryle, to see what should be done next.

Marshall's personal view, that economic debate lies somewhere between resolvable disagreement on technological fact and irresolvable disagreement on religious theology, leads him to look for agreement on what should be done to resolve uncertainties. Hence his own preference, amply demonstrated in the case of wind power, for a systematic step-by-step scaling-up approach—a view which, as he points out, also goes down well with the Treasury.

Chris Sherwell

*The prospects for the generation of electricity from wind energy in the United Kingdom, Energy Paper Number 21 (HMSO, £2.25)

URANIUM

Solidarity and optimism

The Uranium Institute held its second annual meeting in London last week. Chris Sherwell reports

IT HAD been a busy year since members of the Uranium Institute last gathered in the splendid London facilities of the Institute of Electrical Engineers. The uranium cartel controversy had blown up. Anti-nuclear forces had made headway. And a new US nuclear policy had emerged. But if most of the more than 200 delegates who attended last week's meeting came somewhat chastened by the events of the previous twelve months, after two days closeted together they seemed to leave in slightly more buoyant mood.

A renewed feeling of solidarity was perhaps an inevitable outcome, but the sense of optimism that emerged from the usual business of exchanging ideas and informally discussing recent developments was a bonus. That optimism was expressed by Terry Price, the Uranium Institute's secretary general, when in summing up the formal symposium on uranium supply and demand he offered his 'scenario' for the immediate future.

Once it was appreciated that numbers mattered and there was agreement on the facts, he said, the realisation

that there are no easy answers would see the reinstatement of nuclear power to respectability. The current debate would be damped down, a large programme of thermal reactor building would go ahead and the industry's long lead-times would see a determination to push ahead on both fast breeders and reprocessing. The lasting legacies of the present period of uncertainty would be "wholly beneficial", not least the political legacy of a heightened awareness of the need to deal with proliferation.

Earlier the symposium had ranged over the most general and most specific problems relating to uranium and nuclear power. An opening paper examined projected energy requirements to the year 2000, and found that even in a 'world of internal contradictions' (rather than a 'belle époque') there was a large role for nuclear power. Later presentations discussed exploration techniques and factors affecting mining costs. Between these extremes came the most controversial issues—those of supply and demand and of proliferation—and here the real uncertainties arose.

On supply, for example, one paper on the US position contended that unless "drastic action" was taken, "there is not sufficient uranium forthcoming to

sustain a nuclear industry based upon light water reactors". That contrasted with the optimistic assessments of the Ford Foundation report which is apparently the basis of US policy. Another paper on the same subject reveals some of the findings of a US National Academy of Sciences panel; these all indicate serious supply shortages in or beyond the mid-1980s.

Supply questions easily spilled over into proliferation matters. Anthony Grey of Pancontinental Mining suggested that the US emphasis on international safeguards meant that Australia, by using its influence as a major uranium supplier, could lessen rather than increase the risk of nuclear war. Kyoji Mizumachi of the Tokyo Electric Power Company was more critical of the effects of the US anti-proliferation stance: this "drastic change of policy had been a 'great shock' to Japan. And Terry Price himself took the opportunity to criticise an erudite defence from Harvard lawyer Abram Chayes, suggesting that other countries would not alter their energy strategies without being convinced by their own advisers—and the said Price, would be giving cautious advice. One speaker's conclusion sounded most appropriate: at the moment no quantitative conclusion about the impact of proliferation policies on the uranium market can be drawn.

COMECON

Energy supply problem?

The 31st session of the Council of Mutual Economic Assistance (Comecon), held in Warsaw last week, reflected a growing concern of the eight member countries with raw materials and energy. Vera Rich reports

TO DATE, developments in the fields of raw materials and energy have largely been based on the 'unlimited' supplies of the Soviet Union. According to official doctrine, delays in the supply of these resources have been essentially a matter of logistics; so when the smaller Comecon members want immediate access to these resources, they have to contribute to the cost of development.

Now, however, although the speeches in Warsaw still stress the dependence of members countries on Soviet supplies, there is a tacit recognition that the Soviet Union cannot at present meet the total demands of the bloc. While acknowledging the "decisive

importance" of Soviet supplies, the Hungarian Premier, György Lazar, for example, stressed the need "to fully utilise the resources of the member countries"; he noted that Hungary was stepping up the pace of research into fresh domestic resources of fuel and raw materials. And Manea Manescu of Romania announced a massive cutback in the use of hydrocarbons for power purposes, so that they may be devoted primarily to the petrochemical industry.

The simple answer to the energy problem seems to be nuclear power. Vaclav Hula, the Czechoslovak representative to the Comecon Committee for Cooperation in Planning, put forward plans for the development of atomic power plants which would more than double the nuclear capacity of the Comecon bloc in each successive five-year plan. The final communiqué says the "session particularly stressed the importance of the programme for the maximum development of nuclear engineering of the Comecon countries

in providing a comprehensive solution of issues related to the development of the atomic power industry, and a solution to the entire problem of fuel and energy".

The communiqué recommended that an agreement on multilateral international specialisation and cooperation in the production and delivery of atomic power station equipment for 1981–1990 should be concluded next year. No formal reference was made to the potential hazards of a major nuclear programme, and the only reference to the environment was in a list for suggested inter-state conferences that would lead to "constructive business cooperation" with the capitalist countries. The Carter nuclear policy was not, apparently even mentioned.

A major conversion to atomic power has long been a principle of Comecon energy policy, which is not hampered by ecology-minded minority pressure groups. The new resolution, therefore, is not so much a change in policy, as a recognition that it is time to proceed from theory to practice.

IN BRIEF

Windscale contract details

In the second week of the Windscale inquiry in Britain, during the whole of which BNFL's managing director, Mr Con Allday, remained on the stand, further details of the proposed deal with Japan to reprocess spent oxide fuel emerged. Having earlier given sight of the proposed contract, BNFL published a summary of the terms detailing the amount of fuel involved (1,600 tonnes), the Japanese payment of development costs (£150 million) under the 'cost-plus' arrangements, and the value of the contract to BNFL (£400 million).

An official of the French reprocessing firm COGEMA, speaking at the Uranium Institute meeting last week, gave details of French reprocessing plans and listed the conditions attaching to the 'cost-plus' type of contract that COGEMA and BNFL have developed to fulfil "social and political requirements". These were: no financial

risk for the reprocessor in reprocessing foreign fuel; an advance from the utility of its share of the total plant investment in the form of prepayment for reprocessing services; and a contractual agreement, backed by an understanding between governments, giving the option of returning waste to the country of origin for final storage in an agreed form.

Whale quotas announced

At its Canberra Conference last week, the IWC announced the largest cutback in the number of sperm whales which can be caught in the North Pacific. It recommended a complete moratorium on the killing of male sperm whales in the area and set the quota for females at 763; the equivalent figures adopted for this year were 4,320 and 2,800 respectively. As the North Pacific is the main whaling ground for the USSR and Japan, the two countries may find it no longer economic to

keep their whaling activities going in the area. Under the IWC's rules, however, objections to decisions can be raised up to 90 days after the conference and Japan and the USSR are expected to do this.

Although some conservationists thought the cutbacks were as favourable as could be expected, others feared that the IWC's reductions for next year were an indication of mismanagement in previous years: overwhaling had meant that stocks were now very low.

Marshall move

Dr Walter Marshall, chief scientist at the UK Department of Energy, is to leave his post and resume full time work for the UK Atomic Energy Authority. An announcement this week from the Minister, Mr Benn, says this is in view of the UKAEA's significant role in forthcoming important nuclear decisions.

JUDGED by results, the Viking's journey to Mars last summer was an enormous success. Not the least of the wonders was successfully putting down both the landers by programmed automation, virtually blind-fold, among rocks large enough to topple them. These obstacles were too distant to be perceived in the images received on Earth before the descent started. The final 30 kilometres of drop lasted only 5 minutes. Six kilometres above the surface, the parachute blossomed out, unseen in the thin and ghostly atmosphere of Mars. Retro-rockets were ignited that pushed backwards against gravity, the lander settled gently on the surface, and its camera had taken a picture of the terrain before anyone learned of the landing. Automatic instruments chattered out the news by radio waves that took 20 minutes, at the speed of light, to get back to huge dishes, poised 350 million kilometres away in Australia, Spain and California, scooping up the incredibly faint signals.

The news was not of little green men, but of nitrogen, oxygen, krypton and xenon, of the exact temperature, and of a reddish magnetic dust on the surface. But it was the pictures of the barren, rock-strewn landscape with the metal footpad of the lander in the foreground, that spread the tidings most dramatically all over the world.

Incredibly, all the complex instruments were in working order in both landers except for one seismometer. Martian soil was scooped up and tested for life by three procedures.

The first (gas exchange) measured changes in the gas above a sample of soil moistened with a rich culture medium. The Martian soil im-

Mission to Mars



THOMAS H. JUKES

mediately released some oxygen (surprise number one). The second procedure (labelled 'release') looked for production of radioactive gas from a culture medium containing radioactive nutrients. There was rapid evolution of such gas when a fresh soil sample was added to the medium.

The third test shone a light on untreated, unmoistened soil in presence of $^{14}\text{CO}_2$ and ^{14}CO for 120 hours. The

soil was then pyrolysed and the gases that escaped were examined for products of photosynthesised organic matter. The first result was positive, although weakly so. But later experiments showed that preheating the soil to 90° for nearly 2 hours had no effect on the reaction, so the experimenters concluded "... it is unlikely the reaction is biological".

And so the initial feelings of gratification and excitement became tempered with wonder and mystification. We had steeled ourselves for negative findings (a cat without a grin), but we had not expected atypical and perhaps non-biologically positive results (a grin without a cat). The absence of the cat was underscored by negative results in tests for organic compounds in a fresh sample of soil carried out with an extraordinarily delicate 'gas chromatography mass spectrometer'.

The other instruments in the Vikings have sent a wealth of information on Martian climate, geology, meteorology and elementary composition. The North polar cap is now known to be ice. The atmospheric analysis agrees with a concept expressed by one scientist that the cap may be the tip of an iceberg; the rest of the ice is covered with Martian soil.

And so the Earth spins on its orbit delicately poised between the sterile inferno of Venus and the cold and dreary desolation of Mars. We are just the right distance from the Sun to be the planet of harvest, dew and rain; the source and abode of life, unnumbered as the sand.

correspondence

Science and ideology

SIR,—As a typically naive graduate student, I have tended to assume that science should be pursued in a rational and objective fashion and that ideology and political viewpoint have little effect on the scientific truth of a subject. Admittedly, this view stands in contradistinction to the dialectical materialism of Marxism as interpreted by Lysenko, but I have been thoroughly impressed by the advances in human knowledge that have occurred during the past several centuries using Francis Bacon's inductive approach to scientific reasoning.

At any rate, while at the recent annual meeting of the Biophysical Society in New Orleans I noticed that Dr Schwartz had organised a forum 'Racist and sexist implications of sociobiology' (note how the title is free of any ideological predisposition involving connotative and pejorative adjectives). Owing to other commitments, I missed the talk by the geneticist but I did arrive in time to catch the talk by the representative from the Boston Committee Against Racism (which I later decided should be more aptly named the Boston Committee Against Intellectual Freedom).

I had, again in my naiveté, hoped for a rational presentation of the reasons that sociobiology might be on weak scientific grounds. Instead, the talk began with the speaker admitting that he was not an expert in the area and then continuing with the unqualified assertion that the material in Edward O. Wilson's book, *Sociobiology: The New Synthesis* (1975, Harvard Press), was not scientific with the implication that no respectable scientific journal would publish such material. One of his principle objections to the theory was its use of what the speaker termed "anthropomorphism of non-human animals and insects", which, when one cut through the persiflage, meant that he strongly objected to extrapolating observations on hereditary characteristics of insects and animals to man.

It is worth noting that Lysenko used the exact same justification for outlawing medical genetics in the Soviet Union (you should see the verbal persiflage of Marxist rhetoric Lysenko used to 'prove' that human chromosomes could not exist). More mundanely,

some of my rural relatives here in Alabama use a similar rationale to 'prove' that evolution is false—God could not possibly create man along the same tree as other animals.

As the talk progressed, we were subjected to numerous emotive and pejorative terms such as racist, fascist, sexist along with the not-so-subtle hint that anyone who would view the subject from the standpoint of possible scientific validity had to be one of the above. (Reminiscent of the way that Lysenko characterised Soviet biologists who dared believe in Mendelian genetics as "enemies of the people".) The speaker certainly did not believe in a rational scientific approach but preferred to employ connotative, pejorative, obfuscatory, equivocal, circumlocutionary verbal persiflage (*sic*) that, while difficult to follow on a logical level, certainly sounded authoritative and proved even more difficult to refute. Under the circumstances, I can quite clearly see how the Eastern European IQ scare of the early 20th century is somehow related to the scientific validity of sociobiology.

Personally, I have no idea whether or not sociobiology will prove to contain elements of truth. However, I do not believe that one's ideological views are likely to change facts (even though the medieval church suppressed Aristotle's teaching for close to a thousand years because it disagreed with church doctrine). Certainly objecting to 'anthropomorphism' (the speaker's term) solely on the ground that it is objectionable flies in the face of the inductive process on which science is based. The proper approach is to make an extrapolation to man and then design experiments or search for data in an objective fashion that might prove or disprove the hypotheses involved. Unfortunately, I am not optimistic that this is likely to occur given the present political climate not only in the United States but worldwide. I am sure that any data obtained to support either the truth or falsehood of sociobiology, IQ testing or any of the related areas will be denounced by the other side—or in many cases buried under numbers and statistics from which one can, through convolution, draw one's own interpretation of the data (see Matters Arising, *Nature*, 266, 279–281; 1977).

Certainly the fact that the Bio-

physical Society would invite a speaker who was making such an overtly ideological attack speaks poorly for the organisers of the conference. I prefer to listen to my relatives in the rural Deep South discuss how evolution is a Godless bolshevik conspiracy and how Darwin was one of Satan's emissaries. Their tone is admittedly anti-intellectual, but at least it isn't ostentatiously pretentious. If one removes some of the intellectual facade from many of the ersatz scientific debates heard in the areas discussed in this letter, one will find the intellectual content comparable.

Yours faithfully,

WILLIAM O. ROMINE

*The University of Alabama
in Birmingham,
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Israel's President

SIR,—Contrary to the belief expressed (9 June, page 486), the office of President of Israel has not always been occupied by a scientist.

The first President, Dr Chaim Weizmann, was a biochemist. He was the first reader in biological chemistry at the University of Manchester and later became Director of the David Seiff Institute at Rehovot—subsequently renamed in his honour.

The second and third Presidents, Itzhak ben Zvi and Zalman Shazar, were primarily both politicians of the establishment, although ben Zvi did do some sociological research on ethnic minorities among the Jews. Before becoming President, ben Zvi was known as a founder, with David ben Gurion, of the Histadrut—the trade union movement. Shazar for many years was a newspaper editor before entering politics and subsequently becoming Minister of Education.

Ephraim Katzir, the fourth and current President, is a biochemist of international repute. He is fortunate that the duties of office as President also enable him not to neglect his scientific career.

Perhaps the message which should be passed on is 'Biochemists Rule . . . OK!'

Yours faithfully,

B. SAMPSON

*Federation of Zionist Youth,
London, UK*

news and views

Contractile filament organisation, mechanics . . .

from John Squire

FEW people would question nowadays that most muscle types, including vertebrate smooth muscle, shorten by a mechanism involving the relative sliding of two principal muscle filaments, the thin actin filaments and the thick myosin filaments. The surfaces of the myosin filaments are covered with arrays of globular projections (the ATP-splitting heads of the component myosin molecules) and the relative filament sliding is thought to be due to the interaction of these projections (often called crossbridges) with the neighbouring actin filaments. It has been shown that virtually identical actin filaments (except for differences in length and the presence or absence of the regulatory protein troponin) occur in all muscle types and also that the thick filaments, although far from being identical, probably have their myosin projections or crossbridges organised into closely related surface arrays. An inevitable conclusion from these results is that the local crossbridge environments in all muscles are probably rather similar and that the basic force-generating mechanisms must therefore be similar too. Much current research on muscle is therefore aimed at answering two fundamental questions: 'how does the crossbridge interaction with actin actually generate force?' and, less obvious but equally crucial, 'what is the minimum organisation of actin and myosin molecules sufficient to form a viable contractile system?'. This latter question has become even more important in the light of recent fascinating discoveries of the presence of actin and myosin molecules in a bewildering variety of non-muscle cells (see Pollard and Weihing *CRC Critical Rev. Biochem.* **2**, 1; 1974). It now seems evident that many, if not all cells possess the ability to form rudimentary contractile systems involving actin and myosin and possibly that these systems comprise filaments which are formed spontaneously at different places and times as required by the immediate needs of the cell.

From studies of the molecular

arrangements of the ordered arrays of actin and myosin filaments in different muscles it has become apparent that a common organisational feature crucial to muscular contraction concerns the relative polarities of the interacting filaments. Although different muscles may involve slightly different polarity arrangements the structure of a sarcomere in striated muscle (Fig. 1) serves to illustrate the point. Here the myosin filaments are bipolar; the myosin molecules of each half of a filament point in opposite directions. The actin filaments are arranged so that those which interact with one end of a thick filament are of opposite polarity to those interacting with the other end.

Organisation

The organisation of filament polarity has been illuminated by a number of *in vitro* studies. For example it was shown by Huxley (*J. molec. Biol.* **7**, 281; 1963) that synthetic self-assembled myosin filaments with a bipolar structure can be prepared from relatively pure preparations of myosin. Bipolar assembly must therefore be an intrinsic property of the myosin molecules themselves. Similarly, self-assembled actin filaments can be prepared from actin solutions and these have been shown to be polar by labelling them with a preparation of isolated myosin heads (myosin heads which have been cleaved enzymatically from the rest of the myosin molecule). Such 'decorated' actin filaments show a characteristic 'arrowhead' polar structure, (Huxley *op. cit.*; Moore *et al. J. molec. Biol.* **50**, 279; 1970) which shows clearly that

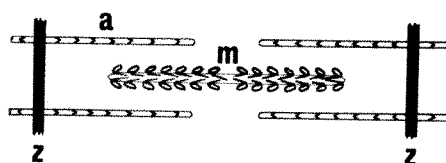


Fig. 1 Highly schematic diagram of the organisation of polarities in a striated muscle sarcomere. The Z-bands (Z) define the ends of the sarcomere. (m is a myosin filament, a an actin filament). Polarity in the thin filaments is indicated by the arrow heads.

the two molecules need to be pointing in the right directions for their highly specific interaction to occur. Polarity organisation must therefore be a crucial requirement for a viable contractile system.

In a highly ordered muscle, such as a vertebrate skeletal muscle, one could speculate that the assembly of myofilaments might well be organised systematically during myogenesis by external factors so that, for example, actin filaments of the correct polarity are laid down at each end of the sarcomere. But it is not so clear that a comparable external mechanism could be involved in organising the filament polarities in spontaneously formed contractile assemblies in non-muscle cells.

Fortunately, in a recent study of actin/myosin self-assembly, evidence has been obtained which reduces the need to postulate an external factor in the assembly process. Hayashi *et al.* (*J. molec. Biol.* **111**, 159; 1977) have described the results of experiments in which globular actin molecules were added to a solution of synthetic myosin filaments to give a molar ratio of 2:1 and conditions were then altered so that the actin molecules polymerised into filaments. The structures which resulted were filament assemblies comprising bipolar thick filaments interacting at each end with thin filaments of opposite polarity; the latter presumably being defined by the myosin crossbridge polarity. These self-assembled contractile units are in many ways analogous to a small part of a muscle sarcomere with roughly six thin filaments interacting with each end of a thick filament. The only thing obviously lacking is a structure analogous to the Z-band to link the contractile units end-to-end.

Myogenesis is much more complicated, since there are many additional proteins in the sarcomere and, among other things, the thick and thin filaments have very precisely defined lengths. But these new results may show how spontaneous formation of relatively simple contractile systems might occur in non-muscle cells. Whatever the specific polarity organisation in the thick filaments this organisation may itself serve as a template for

ordering the polarities of the thin filament arrays. Since some of these non-muscle cells contain α -actinin, a protein also associated with muscle Z-bands, and presumably all the necessary regulatory proteins as well, it seems that most of the ingredients needed for the self-assembly of a simple contractile system may already be known. But one thing which is not clear from the new results is the actual mode of formation of the bipolar contractile units. The possibility favoured by Hayashi *et al.* involves the binding of actin monomers to myosin cross-bridges before actin polymerisation occurs. But the alternative, that actin polymerises and the resulting filaments then interact with the appropriately oriented end of a myosin filament, cannot be ruled out. A useful follow-up to this study would be to decide clearly between these alternatives possibly by determining the state of aggregation of the actin and myosin before polymerisation of the actin.

Crossbridge interaction

Turning now to the nature of the crossbridge interaction itself, equatorial X-ray diffraction evidence from vertebrate skeletal muscles and insect flight muscles has indicated that on activation there is a movement of mass, presumed to be the myosin cross-bridges, towards the actin filaments. Although attachment to actin probably occurs this has not actually yet been proved. Huxley (*J. molec. Biol.* **37**, 507; 1968) took the movement to be largely radial from the myosin to the actin filaments. On this basis the amount of mass transfer in active vertebrate skeletal muscle, calibrated against the transfer in rigor muscle in which actin labelling by 50 to 75% of the available myosin heads probably occurs, is about 50% of the rigor transfer (Haselgrove & Huxley *J. molec. Biol.* **77**, 549; 1973) or up to 80% (Matsubara *et al.* *Nature* **255**, 728; 1975). Cardiac muscle must be the most specialised of all the vertebrate striated muscles; it contracts rhythmically and is not voluntarily controlled. But a recent equatorial X-ray diffraction study of contracting heart muscle (Matsubara *et al.* *J. molec. Biol.* **111**, 121; 1977) has shown that a mass transfer corresponding to that in skeletal muscle occurs in this muscle too. Assuming the crossbridge movement to be radial, about 20% transfer (compared with rigor muscle) occurs during the contracting systolic phase and even during the diastolic phase there seem to be more cross-bridges near to the thin filaments (7 to 9% of the rigor transfer) than in a truly quiescent muscle. Sub-maximal tension generation was said to account for the small systolic transfer (20%)

relative to the 50 to 80% in skeletal muscle. Despite this it seems clear that comparable crossbridge movements occur in all these different muscles. But it remains to be determined what these movements really are. Are they principally radial as suggested by Huxley (see also Haselgrove *et al.* *Nature*,

261, 606; 1976) or would azimuthal crossbridge movements account more satisfactorily for the observations (Lymn & Cohen *Nature*, **258**, 770; 1975)? No doubt this fundamental problem of interpretation will be central to future equatorial X-ray diffraction studies of muscle. □

... and biochemistry

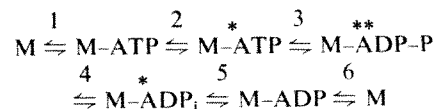
from Howard White

UNDERSTANDING the way in which muscle and other contractile systems couple the production of mechanical work to the hydrolysis of ATP will almost certainly require a detailed understanding of the mechanism of ATP hydrolysis by myosin and actomyosin (the contractile system composed of actin and myosin) and a correlation of the biochemical mechanism with the physical events of the contractile process. It is more or less accepted as dogma that during contraction the heads of the myosin molecules form cross bridges with the actin filaments, which attach and detach in a 'rowing' motion.

The mechanisms of myosin and actomyosin ATP hydrolysis (equation 1) that were outlined six years ago by Lymn and Taylor (*Biochemistry* **10**, 4617; 1971) have been especially useful because they correlated biochemistry with the cross-bridge cycle in a natural way. They showed that ATP binds to actomyosin and dissociates the myosin heads from actin before the ATP is hydrolysed. The overall rate of myosin ATP hydrolysis in the presence of actin and in contracting muscle is several hundred times more rapid than myosin alone because the reattachment of the myosin heads to actin increases the rate of the slow step of the mechanism—product release. The drive stroke in muscle presumably occurs with the return of the cross bridges to the original attached state of actomyosin without bound nucleotide.

Since Lymn and Taylor's work, the mechanism of ATP hydrolysis by myosin (or rather proteolytic fragments of myosin, S1 and HMM, which are soluble at physiological conditions and more tractable for solution chemistry) has been studied in detail. The evidence which shows that there are at least five intermediates involved in the reaction has been recently reviewed by Trentham (*Q. Rev. Biophys.* **9**, 217; 1976).

Both ATP (Step 1 and 2) and ADP (Step 6 and 7) bind to myosin in two-step reactions. The initial weakly bound



nucleotide complexes, M-ATP and M-ADP are converted at apparently identical rates to higher affinity complexes M*-ATP and M*-ADP. The transition of the high affinity complexes is associated with changes in the ultraviolet absorption and fluorescence (the number of stars given to a state gives a rough indication of the degree of enhancement of the tryptophan fluorescence). The major difference between the myosin ATP and ADP complexes is that the rate constant for M*-AXP $\rightarrow$ M-AXP is much smaller for ATP than ADP and hence ATP binds with a much higher affinity ($K_1 K_2 \approx 10^{11} \text{ M}^{-1}$), than ADP ($K_5^{-1} K_6^{-1} \approx 10^6 \text{ M}^{-1}$). The hydrolysis of ATP bound to myosin (Step 3) is rapid and surprisingly reversible, $K_3 = 10$. The rate-limiting step of myosin ATP hydrolysis is associated with the release of phosphate from the myosin thus making M**ADP-P_i the predominant steady state intermediate. The favourable free energy of hydrolysis of ATP in solution is therefore expressed primarily in the difference between the binding energies of ATP and ADP.

This type of kinetic approach is now being used to determine how actin modifies the rate and equilibrium constants of the myosin-catalysed ATP hydrolysis, and in particular to identify the rate-limiting step, and show which intermediates are common for both the myosin and actomyosin mechanisms.

Chock, Chock and Eisenberg, (*Biochemistry* **15**, 3244; 1976), and Sleep and Taylor (*Biochemistry* **15**, 5413; 1976) report that the first intermediate after dissociation of myosin-S1 from actin by ATP undergoes a fluorescence enhancement that is similar to that observed for ATP binding to myosin. Both laboratories mixed the actomyosin-S1 with a high concentration of ATP to ensure that the



change in light scattering due to dissociation of the actomyosin would be complete within the mixing times of their stopped-flow equipment and not complicate the interpretation of the fluorescence change. Although results and interpretation of the two groups differ slightly in detail, both reported that the change in tryptophan fluorescence which occurs after the dissociation of myosin from actin has a similar rate and amplitude to the fluorescence change observed for ATP binding to myosin. Sleep and Taylor were able to show by rapid quenching experiments that the ATP bound to the intermediate did not exchange with the ATP in solution (as would be expected for M-ATP). They dissociated actomyosin with stoichiometric ^{32}P -ATP and found that an excess of cold ATP added before the fluorescence increase had occurred did not reduce the amount of ^{32}P -ATP hydrolysed. It therefore seems most likely that the intermediate first released from actin is $\text{M}^*\text{-ATP}$ and that the fluorescence change occurs during the transition $\text{M}^*\text{-ATP} \rightarrow \text{M}^{**}\text{-ADP-P}_i$.

These observations are especially important, first because they link the change in optical properties of nucleotide binding to myosin with the change in optical properties of nucleotide binding to actomyosin and second, because they show that a change in fluorescence occurs at a step of the reaction that could be associated with the movement of the unattached myosin crossbridge. However, caution is necessary before suggesting that the fluorescence change is an indicator of crossbridge movement. The changes in optical properties observed for myosin and actomyosin are not unusual for the binding of large ligands to protein molecules. Furthermore, there is a conspicuous absence from the muscle literature of a demonstration that ATP induces gross physical changes in the structure of myosin, myosin filaments or myosin proteolytic fragments.

Chock, Chock and Eisenberg (*Biochemistry op. cit.*; 1976) (and for that matter Taylor and myself (*Biochemistry* 15, 5818; 1976) have found that the rate of recombination of myosin-S1-ADP-P_i with low concentration of actin, measured by turbidity, is proportional to actin concentration and equal to the steady state rate of ATP hydrolysis; this would be expected from the Lymn-Taylor model at actin concentrations where the rate limiting step is recombination of M-ADP-P_i to actin. Eisenberg also found that at rather low ionic strength and temperature ($\mu = 10 \text{ mM}$, 4°C) the steady state rate of ATP hydrolysis and recombination of S1-ADP-P_i and actin both reached a maximum of 1.0 s^{-1} at higher actin concentrations, and that the actin and

myosin remain mostly dissociated even at the highest actin concentrations. If a step associated with ADP or phosphate release were rate limiting for actomyosin AT Phydrolysis at high actin concentration, then one would expect that the predominant intermediate would be actomyosin-S1-ADP-P_i; that is, most of the myosin-S1 would be bound to actin. Because the covalent ATP bond splitting was thought to be rapid ($\sim 100 \text{ s}^{-1}$ at 20°C), they reasoned that there must be another step in the reaction, a rate-limiting transition from a myosin-S1-ADP-P_i intermediate unable to bind to actin (the 'refractory state') to an intermediate capable of interacting with actin. However, Taylor (*Biochemistry* 16, 432; 1977) has extended the original measurements of the rate of the covalent ATP bond hydrolysis and found it to be exceedingly temperature dependent. At 4°C the ATP is cleaved at only 3 s^{-1} , a value quite near the maximum rate of ATP hydrolysis, 1.0 s^{-1} , measured for actomyosin by Eisenberg at the same temperature. It is therefore possible that the rate-limiting step could be the hydrolytic step and the refractory state could be $\text{M}^*\text{-ATP}$. Moreover, it is unclear whether the rate limiting step of actomyosin ATP hydrolysis is like myosin in being associated with product release or whether it is the bond-splitting step.

One may quite reasonably wonder how

the rate and equilibrium constants determined in solution can be applied to the highly organised contractile apparatus in muscle. Taylor and I have calculated the "effective standard free energy changes" between each of the intermediates of actomyosin ATP hydrolysis using the equilibrium constants measured in solution at nearly physiological conditions and using as the standard state the concentrations of ATP, ADP, and phosphate normally present in muscle. We found that more than 50% of the "effective standard free energy change" during ATP hydrolysis is associated with the release of ADP and phosphate from actomyosin. Intuitively, it would be expected that the steps with the largest effective free energy changes in solution ought to be the ones coupled to the production of work *in vivo*. However, as has been pointed out in a very elegant theoretical analysis by Hill and Simmons (*Proc. natn. Acad. Sci. U.S.A.* 73, 36, 95; 1976) and Hill and Eisenberg (*Biochemistry* 15, 1629; 1976) restriction of actin and myosin to a given three-dimensional structure could drastically alter the distribution of the equilibrium constants between the biochemical states.

The next stage of this type of work might be to measure the rate and equilibrium constants in myofibrils or muscle fibres where the three dimensional structure and ability to do work is maintained.

New technique for surface crystallography

from John Pendry

THE detailed knowledge of atomic arrangement provided by X-ray and electron diffraction is the cornerstone of our understanding of solids. These techniques have been enormously successful and almost all commonly studied crystals, at least in the area of the physical sciences, have had their atomic coordinates determined. Recently much interest has been focused on surfaces of solids, especially on the adsorption of foreign atoms, an interest driven not only by the problems of understanding these systems, but also by the obvious relevance to more applied areas. The same basic requirements for surface atomic geometry exist as for bulk but the absence of a simple effective technique applicable to surfaces is an impediment to progress. Diffraction of X-rays cannot be used because the weak signal from a single layer of atoms on a surface would be swamped by the bulk signal, and the same is true for high energy electron diffraction. By making use of the increased

scattering cross sections of atoms for low energy electrons (0-200 eV) it is possible to do experiments sensitive to the surface and many simple surface structures have been solved in this way, but the experiments are not easy to perform. At the same time theoretical interpretation is involved, limiting accuracy to the $0.1 \text{ \AA}$ level, and effectively blocks extension of the method to complex surface structures. The search is still on for the definitive surface crystallographic technique.

In recent experiments performed on the Stanford synchrotron radiation project (reported by P. H. Citrin at the March 1977 meeting of the American Physical Society in San Diego; see also Stern *et al.* *Phys. Rev. Lett.* 38, 767; 1977), a revolutionary way of solving surface structures has been demonstrated. In one recent experiment the adsorption of iodine atoms on to a face of a single crystal of silver was studied. X-rays were used but not in a diffracting mode: instead their energy was tuned close to one of the inner-electron energies of an iodine atom. The ejected electron behaves like a radar pulse originating from the excited atom. Surrounding atoms can scatter the out-

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going electron back to the source where the scattered electron wave interferes with the outgoing wave, modulating the probability of electron emission relative to the free atom value. Figure 1 shows a sketch of the process. The following formula describes the modulation to a good approximation:

$$\chi(k) = \frac{I(\text{surface})}{I(\text{free atom})} = \sum_j f_j \frac{\sin(2kR_j + \psi_j + 2\delta)}{kR_j^2}$$

where

$$\frac{\hbar^2 k^2}{2m_e} = \hbar\omega - E_c$$

E_c is the binding energy of the core level, $\hbar\omega$ is the photon energy, R_j the distance to the j th neighbour, f_j and ψ_j the amplitude and phase of scattering by the j th neighbour, and 2δ a shift in phase of the wave, caused by refraction in the potential of the emitting atom. The structure is commonly referred to as EXAFS, standing for extended X-ray absorption fine structure, but it must be stressed that the structure is in fact generated by the diffraction of electrons produced by X rays within the surface. χ can be measured for a whole range of k values by increasing the energy of the input photon, so that χ can be Fourier transformed to pick out the various sine-wave components and hence to find R_j . Some corrections must be applied for the k -dependence of f_j , ψ_j and δ , but these can be calculated quite accurately from theory.

The question remains of how to measure $\chi(k)$. X rays of variable energy can be obtained by monochromating a continuum source. High power X-ray tubes have been used, but by far the best source is a synchrotron or storage ring

which emits very powerfully in the X-ray region. The innermost shell, the K shell, is usually chosen for the experiment and cross sections are sufficiently large for a substantial number of surface atoms to be excited. The probability of electron emission can then be monitored in one of several ways. When the technique is applied to bulk atoms the electron emission probability can most simply be monitored by the absorption coefficient for X rays, but for a surface experiment this method is not sufficiently sensitive. Alternatively the presence of excited atoms can be detected by their eventual decay by way of an Auger process involving emission of a second electron of fixed energy independent of the first electron's emission energy. Sensitive electron detectors are available and the Auger signals can easily be detected. It was in this way that χ was measured for the iodine on silver investigation.

The experiments have shown that it is possible to use the EXAFS technique at surfaces and have confirmed an earlier determination of the structure by electron diffraction. Interpretation of these experiments is sufficiently simple and well understood that ultimately an accuracy of 0.01 Å should be obtainable, an order of magnitude better than previously possible, and in addition extension to more complex structures is feasible. There are limitations to the technique. Only signals from surface atoms of a type different from those in the bulk can be examined. Not all atoms have inner levels suitable for the experiment because the current generation of X-ray monochromators cuts off at long wavelengths leaving the light elements such as oxygen and carbon inaccessible for the moment. Even if an inner shell can be excited, a suitable Auger transition for the monitoring process must be found. Despite these limitations surface EXAFS is the most striking advance in surface crystallography in recent years. □



appears to be due, says Liane Reif-Lehrer of Harvard Medical School, to monosodium glutamate, which is also used in Japanese, Italian and French restaurants. All-American establishments seem to be blameless.

Reif-Lehrer brought the full resources of modern investigative techniques to bear on the problem, and the encoded results were analysed on a computer at the Eye Research Institute of the Retina Foundation. Over 2,000 individuals of all ages were interrogated, including groups of grade-school children in the towns of Needham and Wellesley, Massachusetts; "the socioeconomic situation of these towns was such that these children were likely to have had multiple exposure to Chinese restaurant food". In addition, public notices were issued encouraging sufferers to describe their experiences. To test whether those who exhibited symptoms were more likely to reply to the questionnaire than those who had never suffered, and who therefore put it in the wastebasket, the non-responders were further pursued by post and by telephone; nevertheless an obstinate 3 still refused to answer the questions. A further 22 "did not answer telephone or had terminated appointment". How many of these had suffered nervous breakdowns on account of all the pestering is not recorded.

Among those who gladly volunteered to describe their symptoms, the majority of those susceptible to CRS (about 25% of the total) reported combinations of such commonplace but disagreeable symptoms as headaches and vomiting, diarrhoea and dizziness, sneezing and stomach cramps; not to mention tightness around the face and burning in the upper torso. It is, however, in the list of 36 "other symptoms" that the full horrors of CRS emerge. Depression, hallucinations and an urge to laugh and cry were reported; also excessive perspiration, a feeling of having heavy arms and legs, or "head feels unattached". Less alarm-

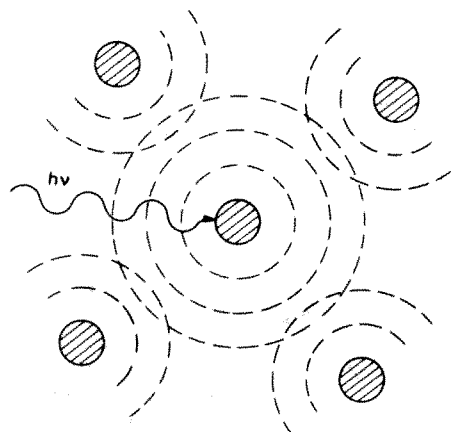


Fig. 1 Electrons emitted by an activated atom are bounced back off neighbouring atoms with a phase lag determined by the interatomic distance and the electron energy.

Advances in Chinese Restaurant research

from a Correspondent

INVESTIGATION of the dreaded Chinese Restaurant Syndrome (CRS) continues to grip the Boston medical research community like a fever. Another comprehensive account of the range of symptoms induced in some of those who (despite it all) still eat Chinese food has just appeared in *Federation Proceedings* (36, 1617; 1977). The list bears comparison with any mediaeval catalogue of bodily ailments. It all

ing were feelings of drowsiness or a "sense of fullness after limited amount of food". Four respondents blamed their Chinese meal for insomnia, while one said it made him feel "high". Another complained of loss of reflexes, making it hard to carry out typing. Others felt paralysed in the neck or backbone, and one unfortunate had a "mental image of a buzzing sound". There was also a wide range of ocular disturbances, from blurred vision to scintillating scotoma (the research was supported by a grant from the National Eye Institute). The most dramatic description, sparing no detail, was "Burst blood vessel and occasional sensation as if pins were being stuck in the eyeball".

All these fascinating data were analysed exhaustively with respect to the respondents' age and sex, though 337 people unaccountably failed to disclose the latter. It seemed that the 26-30 age group was the most at risk. Replies from the 317 children tested were dis-

missed for some reason as "far less reliable than the adult data", but their descriptions have nevertheless the ring of truth; many of those afflicted reported their only reaction as being a stomach ache, while some felt "fat", "stuffed", or "very full" after a Chinese meal. Two children did say that they "felt very funny" and others reported milder reactions, but none described such an interesting variety of bizarre symptoms as their elders, most of whom were faculty members or college students. Perhaps the children's vocabularies were insufficiently developed.

The author ends with thanks to a veritable army of helpers, including five who "stuffed envelopes". Her appetite for symptoms seems only whetted by this wide-ranging exercise, and she says "we hope to provide more definitive data on this matter in the near future". The First International CRS Symposium can hardly be far away. □

Rat readings

If to have a newsletter devoted to one confers the ultimate cachet of respectability in the animal world, the laboratory rat has been unaccountably neglected. A new periodical 'Rat News Letter', published by the UK Medical Research Council's Laboratory Animal Centre now puts the rat on a par with several of its laboratory companions. The purpose of the newsletter, which is intended to appear twice a year, is to serve as a medium for the informal exchange of information on all aspects of the biology of the laboratory rat, but with special emphasis on the use of genetically defined rats in biomedical research.

The first issue announces the discovery of an athymic nude mutation which occurred spontaneously in the hooded rat colony at the Rowett Research Institute. This mutant is being bred at the Laboratory Animal Centre and will probably begin to become available in small numbers by the end of the year.

The vexed question of nomenclature for the alloantigenic loci is discussed and the issue also contains contributions from research establishments throughout the world giving details of their interests, and holding of strains, stocks and mutants. The editor is Dr Michael Festing, MRC Laboratory Animal Centre, Woodmansterne Road, Carshalton, Surrey SM5 4EF to whom contributions for publication and enquiries about subscriptions (£5.00/\$10.00 for four issues) should be sent.

Phobos and Deimos, the moons of Mars

from David W. Hughes

A discussion meeting to review our present knowledge of the Martian satellites was held by the Royal Astronomical Society in London on 13 May, 1977, under the chairmanship of Dr G. E. Hunt of the Meteorological Office.

AUGUST 12, 1977 is the hundredth anniversary of the discovery of Phobos and Deimos and meetings are being held in London and Washington to commemorate this event. The discovery was made by Asaph Hall, an astronomer at the US Naval Observatory, Washington DC, using their new 26-inch refractor at the old observatory down by the Potomac river. In 1877 this was one of the best telescopes in the world. Hall started his search in early August, his first few nights being unsuccessful. Discouraged, he was all for giving up but his wife (*née* Angeline Stickney) urged him to spend a few more nights at the telescope and on the 12th he discovered Deimos. While confirming this observation Hall discovered Phobos on the 17 August, the two new satellites being announced on 19 August. Hall named them Phobos (fear) and Deimos (terror or panic) after the horses that pulled the chariot of the god of war in Greek

mythology. Interestingly the two Martian moons had been discussed in the literature before their discovery. Johannes Kepler (1571-1630), a great lover of mathematical progressions, had decided that as Earth had one satellite and Jupiter four (only the Galilean satellites were known at that time) then Mars, the planet in between, should have two. Voltaire and Swift mentioned them in *Micromegas* and *Gulliver's Travels*, Swift in 1726 going so far as to predict the correct values for their orbital revolution periods.

Hall observed Mars during a very favourable opposition when the distance between Earth and Mars was at a minimum (~ 0.57 a.u.). At their greatest elongation from Mars the two satellites are only 22 and 56 arc s from the centre. Even here it is extremely difficult to pick them out from the halo of scattered light that surrounds the telescopic image of Mars. The fact that they are about 3×10^3 times fainter than Mars does not help much either.

At the London meeting D. Pascu (US Naval Observatory) discussed Earth-based observations of the satellites with special reference to the accidental and systematic errors inherent in positional observations. Before 1941 visual measurements were made of the distance between the satellites and of the angle the two subtended at the centre of the planetary disk. The rapid motion of the satellites and the problem of estimating the disk centre, added to

problems of visual astigmatism, made these measurements very difficult. Photography has been used since 1941, small circular or lozenge shaped nicrome filters deposited on optically flat glass plates being used to equate the brightness of the planet and satellite. This technique obviously reduces the personal error and with exposure times mainly between 10 and 20 s the satellites do not move very far during the exposure time. Photography thus reduces the positional error from about 0.6 arc s to less than 0.1 arc s.

Photometric observations are greatly hampered by the logarithmic intensity gradient of the planetary halo and only integrated results over the whole satellite disk are possible and these only to ± 0.1 magnitudes. Pascu concluded by saying that spacecraft pro-

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vided positional results comparable to the best Earth based astrometric observations. The sparsity of the spacecraft data is ample justification for the continuance of telescopic observation.

Orbits

G. A. Wilkins (Royal Greenwich Observatory) discussed the orbits of Phobos and Deimos making special reference to the way in which these are varying with time. Deimos orbits Mars in 30 h 18 min and Phobos in 7 h 39 min, Phobos having the strange distinction of being the only satellite in the Solar System which orbits its central body faster than that body spins on its axis. Looking at the satellites from the surface of Mars Deimos would rise in the East and set in the West just like our Moon does but Phobos rises in the West and sets in the East. There are two other strange things about the orbit of Phobos. First its mean distance from the centre of Mars is 9,400 km whereas the Roche limit is about 8,700 km (any fluid body getting closer to Mars than 8,700 km will be pulled apart by differential tidal forces). Second, it is getting closer. This automatically increases its mean orbital velocity V , Sharpless (*Astr. J.* **51**, 185; 1942) concluding that $\Delta V/V$ per period was about 8×10^{-12} . This secular acceleration could be caused by a resisting medium in the region of the satellite orbit (the outer remnants of a Martian atmosphere?) or be a consequence of tidal friction, the absence of seas on Mars however requiring this to be a body tide. The Soviet astrophysicist Shklovskii in the 1950s favoured the former explanation postulating that the large $\Delta V/V$ was due to the low density of Phobos; he suggested a density of $10^{-3} \text{ g cm}^{-3}$. Shklovskii astounded the scientific world by suggesting that Phobos was in fact a hollow, artificial satellite produced by an advanced Martian civilisation.

Wilkins was not convinced that present-day analysis of observational data indicated that there was any secular acceleration taking place. Spacecraft data seemed to indicate that it was at least a factor of three smaller than that deduced by Sharpless. Even at this lower rate Phobos would crash on to Mars in about 10^8 years assuming of course that it was not pulled apart into a myriad of rocky pieces on coming closer than the Roche limit. Such a disruption would give Mars a ring system like Saturn and Uranus.

Spacecraft observations of the surface features of the Martian satellites were discussed by Jo Veverka (Cornell University) (see also *Scient. Amer.*, February 1977). In 1971 Mariner 9 obtained 29 views of Phobos and 9 of Deimos with an average resolution of 200 m. Surprisingly both satellites were

found to have almost identical shapes, that of triaxial ellipsoids, Phobos having principal diameters of 27, 21 and 19 km, Deimos of 15, 12 and 11 km. Why this is so is still somewhat of a mystery. However, Don Gault (Ames Research Centre) found that high velocity impacts on spherical targets produced a crater at the impact point, spalling on the opposite side of the sphere and often the complete removal of the entire outer layer of the target. The inner core left behind had a shape surprisingly like that of Phobos and Deimos. Both moons rotate synchronously with their orbital periods and their long axes pointing directly at Mars.

View from Viking

The moons are not the same colour as Mars. Mars is red, they are blackish-grey, indicating that the surfaces have different compositions. Albedo and spectral reflectance measurements indicate that the satellite surfaces are made of C1 carbonaceous chondritic material similar to the Orgueil meteorite and the asteroid Ceres. This material has a density of about 2.0 g cm^{-3} . Crater counts indicate that the surfaces are at least 2×10^9 yr old and Veverka concluded that the satellites must have been formed in the asteroid belt and have moved to Mars later on. The Viking spacecraft has approached as close as 100 km to the satellite surface and has obtained pictures with a resolution of about 10 m. These pictures reveal curious rill-like fracture patterns around Stickney, a 10 km diameter crater on Phobos. (This crater has a diameter about 40% the maximum diameter of Phobos itself!) These could be failure cracks produced in the rocky satellite when the crater was formed. Crater chains have also been found, these being parallel to the orbital plain (which is also the equatorial plain of the satellite). These could be formed by secondaries blown off the satellite by impact events, these secondaries having velocities lower than the escape velocity from the Martian gravitational field. After orbiting the planet for a time (that can be as long as 50 yr) some of these fall back on to the satellites, forming secondary craters. The surface also contains peculiar groove-like striations, these forming segments of a series of small circles which are all perpendicular to the Mars-satellite line. Tidal mechanisms are being considered as a possible source of these.

W. Michael (Langley Research Centre) discussed the changes induced in the orbit of the Viking space probe by its close approach to Phobos. These indicate that Phobos has a mass of $1.1 \times 10^{19} \text{ g}$ (about 2×10^{-8} the mass of Mars). The uncertainty in this value is

about $\pm 15\%$ and coupled with a volume which is also known to $\pm 15\%$ this gives a density with a probable value between 2.1 and 2.2 g cm^{-3} and an error of about $\pm 20\%$.

The problem of the origin of the Martian satellites was discussed by M. Woolfson (York University). Theories are legion—a usual case when there is not enough available evidence. Most have the two satellites originating at the same time mainly because their orbital orientations are similar, as are their photometric characteristics. They are thought of as being planetismals left over from planetary formation, condensed filaments produced by collapsing protoplanets, captured asteroids that crawled into the inner Lagrangian points then lost energy by tidal friction giving them more stable orbits, fragments from a disrupted asteroid that passed within Mars' Roche limit, fragments from a collision between two asteroids that took place within Mars' gravitational sphere of influence or fragments produced by collisions between protoplanets or between protoplanets and Jupiter, these collisions resulting in all the asteroids and terrestrial planets and their satellites.

So a hundred years has seen a vast increase in our knowledge of these two fascinating satellites. One of the bonuses of sending a spacecraft to Mars is that you have these asteroid-like bodies on hand for investigation if Mars becomes blotted out by dust storms. Viking went within 100 km, let us hope that in the near future a spacecraft will be sent to land on Phobos or Deimos to see what their surfaces are really like. □



A hundred years ago

THE annual meeting of the Institution of Mechanical Engineers opened on Tuesday at Bristol. Mr. T. Hawksley, C.E., in his opening address, said it was the duty of the government to adopt such timely measures as would secure to us the paths of the ocean for our food inwards and our manufactures outwards. He deprecated the building of enormous and unwieldy floating castles, and advocated the construction of a fleet of swift, light, well-engined ships, equally capable of sailing or steaming. He thought the extreme action of some of the working classes the cause of England's trades going abroad.

From *Nature* **16**, 26 July, 256; 1877.



Prospects in oceanography

At a time when science in general has lost its magic, the science of the earth—and especially of its mysterious ocean—has been increasingly appreciated for its combination of difficulty and importance.

Plate tectonics helped a lot by providing a unifying hypothesis which could be readily grasped, and which justified the investment in earlier observations at sea, especially of the magnetic field. The deep drilling programme, now IPOD, has been a triumph of science, technology and organisation. The physical oceanographers have learned how to make direct measurements of ocean currents and discovered that the deep ocean is quite different from what they expected. There are eddies which may well be the marine equivalent of atmospheric depressions, longer lasting but smaller and slower. If they resemble atmospheric depressions to the extent of dominating the poleward transfer of heat they will be vital to our understanding of climate and climatic change. Marine biologists can now unambiguously sample macro-plankton in the deep ocean; zoogeography is becoming a quantitative science at last. Marine chemistry is now a subject in its own right.

Much of this progress has been made possible by instrumental development and skilled engineering. We have exchanged our sextants for satellites, we can dive into rift valleys, we can image the earth from space and make micro-photographs at fantastic magnification. And yet we have only just started to understand the ocean; it remains dark, mysterious, enigmatic, the last great pit of ignorance on our planet. There are simple-sounding questions to which we cannot yet give satisfactory answers. An engineer designing an offshore structure may feel that we should know how surface waves are generated by the wind. A forward-looking fisherman might expect us to know about the life-cycle of krill. A climatologist might think that we should know how much heat the Gulf Stream transports.

Oceanographers used to form a small group of rather eccentric, half-scientist, half-seaman explorers. Everybody knew that the ocean provided a support for ships, a source a food, a sink for waste and a regulator of climate, but it wasn't thought that science could help the engineers much. In those days, not so long ago, anyone making a long series of wave-observations would have been accused of wasting taxpayers' money. Now he is asked why he hadn't made many more.

There is an inevitable mismatch between the timescale of marine research and of the practical problems to which it can contribute. Environmental problems arise unexpectedly; stamping them 'immediate' cannot reduce the time it takes to make the necessary observations or get some fundamental understanding. Oceanographers must, therefore, gather as much basic information as they can: they cannot predict, any more than anybody else, what problems will come up so they must necessarily work on a variety of problems, trying to connect them into a self-supporting generality from which special cases can be sensibly studied.

The money spent on such basic research is perhaps analogous to a life assurance premium to guard against an inevitable contingency rather than to react to a particular, predictable event. It is generally desirable to pay one's assurance premium regularly and over a long period. Marine research is a bit too long-term to suit most of its customers, to use the present jargon. Perhaps it needs patrons rather than customers; in the long run, as Lord Rothschild probably appreciates, it is selective patrons who get rich.

A few problems are, however, so long-term as to be foreseeable. One concerns the disposal of high-level radioactive waste. It might be placed on, or preferably in, the sea floor; a study of the effects is clearly essential. Fortunately much relevant information and expertise is already available, thanks to an investment in basic research, so progress can be made fairly quickly, which in this context means in a decade or two.

Another long-term problem is climatic change. Man is vulnerable to relatively small changes in his environment; there is growing concern that we know so little about the mechanism of climatic change, that we cannot assess whether, or to what extent, man's activity contributes to it. If anything is clear it is that the ocean is an important component in the system—the pioneers of air-sea interaction and of ocean circulation studies weren't frittering away the taxpayers' money after all. There is a lot left to do—and it will take a long time.

Long-term, the outlook for oceanography seems good. The short-term gloom stems from a basic incompatibility between an indivisible ocean and an increasingly nationalistic world, which is offsetting what some find the main attraction, the combination of personal effort with international cooperation. Sensible legislation is clearly desirable and able scientists and lawyers are working on it. But it does not seem likely that United Nations Conferences on the Law of the Sea can do much to create the goodwill needed, whatever is finally agreed. The badly needed bridge between politics and science can only be filled by the slow process of education. It may well be that training and mutual assistance in marine science is the most valuable contribution some mature oceanographers can now make. There is a growing risk that marine research will be slowed down, not so much by lack of access but because the bright young people on whom progress depends will prefer a branch of science where freedom to make observations does not depend on unpredictable and unexplained bureaucratic decisions.

Some may argue that a check to the progress of marine research would be a justified casualty of peace, a transient on the way to a new world order. It seems much more likely that oceanographers will be in the lead.

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Developments in the Law of the Sea

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In this and the following article developments in the law of the sea are described and their impact on marine research is considered. Agreement seems close on much of the negotiating text concerned with coastal and economic zones. But beyond, on the sea-bed in deep water, there are still many difficulties to be resolved. Is the outlook for scientific research therefore bleak? It is argued that if scientists come to terms with the new order, prospects for research in the economic zone and beyond could well be bright.

United Nations Conferences on the Law of the Sea and Associated Meetings

First UN Conference on the Law of the Sea (UNCLOS), Geneva, 25 February–27 April 1958.

Second UN Conference on the Law of the Sea, Geneva, 17 March–26 April 1960.

Ad hoc Committee to Study the Peaceful Uses of the Sea-bed and the Ocean Floor beyond the Limits of National Jurisdiction. (Established by UN General Assembly resolution 2340 (XXII) of 18 December 1967.) Three sessions, New York and Rio de Janeiro, March–August 1968.

Committee on the Peaceful Uses of the Sea-bed and the Ocean Floor beyond the Limits of National Jurisdiction. (Established by UN General Assembly resolution 2467 (XXIII) of 21 December 1968.) Eleven sessions (plus additional meetings), New York and Geneva, February 1969–August 1973.

Third UN Conference on the Law of the Sea. (Established by UN General Assembly resolutions 3029A (XXVII) of 18 December 1972 and 3067 (XXVIII) of 16 November 1973). First session (administrative matters only), New York, 3–15 December 1973; Second session, Caracas, 20 June–29 August 1974; Third session, Geneva, 17 March–10 May 1975; Fourth session, New York, 15 March–7 May 1976; Fifth session, New York, 2 August–17 September 1976; Sixth session (scheduled), New York, 23 May–8 (or 15) July 1977.

IN the latter half of the twentieth century the strategic significance of the ocean has substantially increased as a result of the relative invulnerability of the submarine-based deterrent. At the same time, the incidence of violent conflict over purely ocean-related issues has been quite low. But, since the first attempts to codify the Law of the Sea under the auspices of the League of Nations in 1930, there has been a dramatic increase in non-violent conflict over ocean resources, both living and non-living.

The most contentious issue of the period 1920–60 was the limit of the territorial sea as it pertained to jurisdiction over coastal fisheries. This, in turn, was linked to the issue

of freedom of passage through straits used for international navigation since such passage was seen to be threatened by the extension of coastal state jurisdiction over fisheries. In 1968–69, the major problem in the UN Sea-bed Committee was the question of limits to the continental shelf and jurisdiction over the ocean floor beyond the shelf. In 1973–77 at the Third United Nations Conference on the Law of the Sea (UNCLOS III), the shelf and sea-bed remain contentious issues but the number of controversial problems has proliferated. These include the exclusive economic zone concept, straits used for international navigation, archipelagos, islands, scientific research, ship-generated pollution and a host of other things.

The primary function of the law of the sea has always been to arrive at some balance between the exclusive interests of a few states relative to any particular use of the ocean in various spatial locations and the inclusive interests of the rest of the world system. Since 1930 the search for a globally acceptable balance has been posed increasingly in terms of choosing between the inclusive interests of free navigation and the exclusive interests of extending coastal state control over living and non-living resources in wider areas of the water column and continental margin adjacent to their coasts. It is estimated that the focal point of the current negotiations, the 200-mile exclusive economic zone, would result in an additional 35%–37% of the landmass of the planet passing under national control.

The conferences of 1958 and 1960 differed from that of 1930 in the number and type of participants. Twice as many countries (86 and 88) participated in the latter as in the former (44). Expectations about the possible contributions of marine technology and ocean resources to national development seem to have been significantly stimulated at this time. In 1974 participation in UNCLOS III became almost universal with 137 participating countries, out of the 149 invited. In 1976, the number of participants increased to 156. The alignments in the UN Sea-bed Committee from 1968–73 and in UNCLOS III itself were no longer those of the General Assembly but resembled the alignments of the United Nations Conference on Trade and Development (UNCTAD) more and more. In doing so they foreshadowed Assembly politics of the 1970s with the emphasis on the confrontation between advanced industrial and developing countries. This was clearly the most complicated, and perhaps the most difficult, law-making conference the United Nations had ever hosted. There were many more countries represented than ever before; consequently many more interests had to be reconciled. Many of these interests seemed mutually exclusive. Given the primacy of the North–South confrontation, no single group seemed capable of providing effective global leadership.

Technical experts, though in attendance, were tightly controlled by their delegations. They were not allowed to have any independent role as in 1958, since science itself had come under heavy attack. There was no longer any trust and repeated assertions about the necessity of facilitating disinterested scientific investigations of the world ocean

were consistently denied. This was a fight to redistribute ownership and control over world ocean resources and science was seen to be a weapon in this fight.

States differ both in the variety of ocean-related capabilities they command and in the marine biogeophysical conditions which they face. These differences are of particular importance in explaining much of the political behaviour and outcomes in the negotiations. But, in addition, the process and the results of negotiating are very significantly affected by the dynamics of conference diplomacy in global settings. These dynamics include the need to build and maintain 'winning' coalitions as defined by the majorities in the Rules of Procedure, the method of arriving at decisions, the extent to which purely ocean-related issues are affected by considerations external to the oceans, and many other factors.

Emergence of a new regime for the oceans: content and process

Content: The heart of the new regime is contained in two documents: the Revised Single Negotiating Text (RSNT), Parts II and III. Although a few issues remain to be resolved, the outlines of the new law of the sea are in fact quite clear. The situation relating to the sea-bed beyond national jurisdiction, however, presents grave negotiating difficulties and threatens the whole conference with official failure, that is, no treaty. Consequently, the RSNT, Part I, is at the moment extremely controversial.

The basic ingredients of the package in the RSNT, II and III, are as follows. (1) A 12-mile territorial sea with separate regimes governing innocent passage and passage through straits used for international navigation. (2) An exclusive economic zone of 188 miles beyond the outer limit of the territorial sea wherein the coastal state exercises sovereignty over living and non-living resources, exclusive jurisdiction with respect to other activities like scientific research, and jurisdiction with respect to yet other activities like preservation of the marine environment. (3) The coastal state exercises sovereign rights over its continental shelf, which is defined as either the continental margin (including the shelf, the slope and the rise) or 200 miles, whichever is greater. (4) Archipelagic states may draw straight baselines connecting the outermost points of the outermost islands and drying reefs, before delineating the outer limits of the territorial sea, economic zone, and continental shelf. (5) Island states may extend jurisdiction as described but "Rocks which cannot sustain human habitation or economic life of their own shall have no exclusive economic zone or continental shelf" (Article 128, paragraph 3).

The RSNT, III, is itself divided into three parts. The first part deals with preservation of the marine environment and contains primarily very general obligations for states relating to pollution from land-based sources, from or through the atmosphere, and by dumping. The most controversial section (VII) is contained in Articles 23-42 dealing with the enforcement of these provisions over ships and particularly over vessels operating within the economic zones of coastal states. The source of controversy is that the issue is linked to the status of the economic zone as it affects navigation and therefore the security interests of particular maritime states. In a similar fashion, the part on marine scientific research also has broader implications but the specificity of coastal state control is greater. There are, however, some incompatibilities between the approaches adopted in Parts II and III.

With respect to the sea-bed issue, the major points of controversy are as follows. (1) The basic principle governing

exploitation of the area and its resources. The Group of 77 insists that there be only two modalities: either the International Authority directly and exclusively exploits the resources and/or the Authority exploits in conjunction with other contracting parties via the mechanism of joint ventures. On the other hand, the advanced industrial countries insist that a third modality be allowed, that is, exploitation by contracting parties alone. (2) The detailed rules and regulations governing the relationships between the Authority and contracting parties. (3) The structure, functions and powers of the International Sea-bed Authority and, in particular, the method of making decisions within the Council. (4) The method of financing the Enterprise which is to be the operating arm of the Authority. And (5), the question whether the Authority is to be given the competence to set its own resource policy; this implies the capacity to impose production and price controls on contracting parties.

Process: From the first substantive session in 1974, the Conference has suffered from the problems of size and complexity incurred in attempting to negotiate an agenda of more than 100 issues simultaneously among 137-156 nation-states. These difficulties have been aggravated by the Rules of Procedure which emphasise the necessity of achieving consensus, the dynamics of global conference diplomacy which are often at odds with the distribution of interests in the substantive problems being negotiated, and the considerable natural inertia which such a social system brings in its train.

Even during the preparatory phase (1971-73), it was clear that there would be two major overlapping dimensions to the conflicts which would arise in the Conference. The first, already mentioned, would pit developing countries against advanced industrial countries. The second, however, would cut across the former by pitting all landlocked and geographically-disadvantaged countries against those coastal states which stood to gain most from the exclusive economic zone concept. The combined effect of these two factors was to increase the probability that no coalition would actually have the votes to achieve a two-thirds majority and therefore significantly to delay the emergence of a treaty.

The second major problem of the negotiation process at UNCLOS III concerns the sea-bed issue in Committee I. This was in reality a four-dimensional problem. The first dimension was that only 20-30 countries actually had substantive interests at stake in the negotiations. For the remaining 100 or so delegations the symbolic issues were paramount and this was the issue on which the North-South confrontation was at its most extreme. The second dimension is represented by a group of states, led by Algeria, seeking to make this issue into a central issue of the New International Economic Order. They were ultimately successful in this strategy and were significantly aided in doing so by the third dimension: a very serious internal split within the US delegation between the Departments of the Treasury and Interior over the line that would be followed. The fourth dimension was an idiosyncratic one but very significant, nonetheless, since it involved a severe personality conflict between the chairman of Committee I and the chairman of Committee I's Working Group. All of these dimensions affected the negotiations in important ways which we do not have the space to describe in detail.

One final point should be made about the process of negotiations in Committee I. As a result of the scope and technical complexity of the issues being dealt with, each major issue was negotiated separately rather than in a total package as in Committee II with the economic zone and transit through straits. Consequently, the same confrontations occurred at every stage and increased the difficulties of reaching agreement.

Probable outcomes and their effects

Will the Conference be able to produce a treaty or not? This depends primarily on whether either the advanced industrial countries or the Group of 77 capitulate on the major issues of access to and control of the international sea-bed area. If the United States capitulates, it is not clear at the moment that either the EEC countries, Japan, or the US Senate will concur. If there is continued stalemate, the Conference will be by-passed by unilateral action of the US Congress probably in late 1977 or early 1978. In that event, undoubtedly the EEC countries and Japan will follow suit.

While the sea-bed is the primary problem for the next session (May–July 1977), there are still three difficult issues remaining in Committees II and III. (1) The status of the economic zone or whether the zone is to be declared waters under national jurisdiction with specified easements for coastal states or vice-versa. If neither is the case, the problem is whether the zone is unique. The underlying problem concerns the residual rights of navigation within the zone and their significance for the defence establishments of the USA and USSR. (2) The problem of coastal state control of scientific research undertaken within its economic zone. (3) The continued, intense opposition of the Group of landlocked and 'geographically-disadvantaged' States to the articles establishing the economic zone. While

these are difficult problems, there are indications that solutions can be found.

If the Conference breaks down over the sea-bed issue, the result is likely to be continued controversy over the RSNT, I, but a high degree of acceptance of the provisions contained in the RSNT, II and III. Re-revised versions of these are likely to be presented as formalised President's Texts outlining the new regime for the oceans. Already, in the large number of unilateral extensions of coastal state jurisdiction of 200 miles, most of them are obviously patterned after the RSNT, II.

What then are the likely substantive effects of such a regime? The most sweeping changes would relate to fisheries wherein almost all the world's demersal species currently fished and about 60% of tuna and tuna-like species will pass under national control. The effects on management of tuna in the short term will be grave since control over the stocks will be fragmented. The stability of the regime for navigation will be in question as far as the issue of coastal state control over ship-generated pollution in the economic zone is concerned. The scientific research activities of advanced maritime countries, and particularly of the USA and USSR, in the economic zones of other coastal states are likely to be adversely affected. Finally, it will become increasingly more clear that the economic zone will be a multi-purpose zone for which a capacity to provide co-ordinated management across all activities should be developed.

Implications of the Third United Nations Conference on the Law of the Sea for marine scientific research

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THE Third United Nations Conference on the Law of the Sea (UNCLOS) and its predecessor—the United Nations Committee on the Peaceful Uses of the Sea-bed and the Ocean Floor beyond the Limits of National Jurisdiction—have, for the last 10 years or so, from a starting point of Arvid Pardo's 'Common Heritage of all Mankind', been steadily trying to reach agreement as to how the resources of the oceans beyond the limits of the old territorial sea—a cannon shot range of 3 miles from the coast—can be divided equitably between the 150 odd sovereign member states of the United Nations.

Numerous other problems have arisen along the way which have required investigation and solution but the resources, both living and non-living (mainly the latter), are the sole real reason for the whole exercise.

This monumental task which is as yet far from complete, seems to be moving at a desperately slow pace but it should be remembered that it is indisputably the greatest single attempt ever made to develop an entirely new international legal regime for a highly complex subject area having numerous facets and linked interests, all of which have to be taken into account. When complete the resulting Convention will cover the waters of the oceans, the sea-bed and the subsoil beneath, over an area covering more than 70% of the surface of the globe. It should be realised that the First UN Conference on the Law of the Sea (the Second

was limited in scope and was a failure) was, in essence, an attempt to codify existing traditional laws that had developed haphazard over the years, but that this is an entirely different type of exercise that cannot be carried out in a hurry if the final result is going to be acceptable to a majority of the countries of the world.

It has been said that the Conference is an attempt to avoid the development of a situation similar to the African land grab of the nineteenth century but in the event, this is exactly what has occurred but with more contenders for the prize and no indigenous peoples to cause difficulties in later years.

The preceding article describes the way in which the Law of the Sea has developed, so no attempt has been made herein to identify the various draft articles and discussions dealing with marine scientific research. But, there are a number of basic agreements that have now been reached and are certain to become generally accepted law in the next few years whether or not an acceptable Convention is forthcoming from the Conference; the likely effect of these on the future of the marine sciences is considered below.

Crisis in marine research

It cannot be denied that fundamental marine scientific research and much applied research of vital importance to developing as well as developed countries, is now facing a

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crisis quite unlike that ever faced by any other science. Ocean processes and living resources do not respect boundaries arbitrarily imposed by man, and scientists are finding that their floating laboratories can no longer be sited where they are needed, nor do they have unrestricted access to areas where they can collect the data that are essential for their research work.

This is inevitable during the transitional period, that is, the duration of the Conference, and it will doubtless cause a temporary withdrawal to accessible areas—to waters under the jurisdiction of like-minded coastal states and to the deep oceans, though the legal status to be accorded the deep ocean floor and subsoil is still under discussion.

There is every indication, however, that coastal states have been stimulated by the Conference on the Law of the Sea and the number of enquiries and requests from developing coastal states for help in building up their marine science infrastructures is growing to flood proportions. Countries are turning in increasing numbers for direct help by bilateral aid and to such bodies as Unesco, OECD and the EEC.

In the long term therefore, it is already abundantly clear that there will be no insurmountable restrictions on the marine sciences. The length of the present hiccup will depend entirely on the perception of scientists in the limited number of countries with developed and sophisticated marine science infrastructures, and their willingness to help other coastal states to develop their expertise and capabilities as part of their on-going marine science programmes, and not as a separate exercise which is always the responsibility of another government department.

Economic zone and beyond

The great majority of participants at the Conference are now agreed (as noted in the preceding article) that the territorial sea should be increased in width to 12 miles and that there should be an economic zone of 200 miles width (from the coast) over which coastal states would virtually have complete sovereignty so far as living and non-living resources are concerned. Furthermore, where the geographical continental shelf extends more than 200 miles offshore, the coastal states will still have the same sovereignty, though a proportion of the profits from exploitation of resources in this extended area may have to be used for the benefit of developing countries. The single most important matter of concern to marine scientists is the almost unanimous wish of the developing countries to insist on scientific research and exploration in these areas being placed in the same category as exploitation of resources, an activity that can only be carried out with the consent of the coastal state. This attitude is due to the not unfounded belief that knowledge of the existence of resources leads to a position of power and a very well founded fear that research ships do not always confine their activities to the project declared and may be collecting data detrimental to the coastal state, coupled with the fact that it is well known that military and commercial interests are in most countries given precedence over those representing scientific research.

The 200-mile width chosen for the economic zone is an arbitrary figure based originally on claims made (and still maintained) by a number of South American countries for the width of their territorial sea. This had nothing to do with the geographical concept of the continental shelf (which had been the basis of the rights of national jurisdiction contained in the 1958 convention, on the principle that the continental shelf is an extension of the continental land mass) but rather was based on the economic need of developing countries on the west coast of South America to lay claim to what was then developing into the greatest fishery resource in the world.

There is no question, however, but that the deployment of scientific research ships in the strip of ocean 200 miles in width from the coast and beyond that distance to an as yet undefined limit which will certainly be in the deep ocean, will be firmly under the jurisdiction of the coastal states. The most likely contender for this outer limit seems to be the outer edge of the continental rise but this is so ill-defined in most oceans that it will remain a theoretical limit rather than one that can be plotted on a chart, for many years to come.

Where the limits can be drawn in without too much difficulty, and future jurisdiction is reasonably clear, is in the regional seas, in particular the North Sea, Baltic, Mediterranean, Caribbean, South China Sea, etc., where the whole sea will virtually be under the jurisdiction of the coastal states.

In the Mediterranean, for instance, this situation has already been recognised quite clearly by the coastal states of the region, and they have retained for themselves the right to formulate all policy regarding research on the marine environment being developed under the auspices of the United Nations Environment Programme (UNEP). This does not, however, prevent states from outside the region, in particular developed countries with research ships and appropriate expertise, participating in the implementation of such research projects.

Prospect for more cooperation

At first glance this might seem to mean that the only research work that will be permitted in such a region and off the coasts in the open ocean, will be that having immediate application to the problems and interests of the coastal states and, as many of these states will be developing countries with little interest in sophisticated research, the outlook may superficially look bleak for fundamental and other scientific research work having wider implications than a purely local interest and application. But this ignores the basic bargaining power in the hands of the developed countries of trained manpower and ships which, if used properly, will allow both parties to obtain what they need: the developing countries—training and education in the marine sciences, experience and strengthening of their infrastructures, and the developed countries—the right to work in areas of scientific interest. Such an arrangement will only work if each party recognises the interests of the other and is prepared to cooperate closely to their mutual benefit.

This calls for three quite separate types of international education to meet the changing situation.

- (1) Education of scientific administrators and decision makers in developing coastal states to enable them to understand the peculiarities inherent in the administration of marine science.
- (2) Education of senior scientists and scientific funding agencies in developed countries to get them to face up to the changing situation and abandon their rearguard action for freedom of scientific research and separate definitions (and therefore regimes) for fundamental and applied research.
- (3) Education and training of young marine scientists in developing countries to build up the national infrastructures and know-how in these countries, so as to educate out the suspicion now so rife in the UN Conference on the Law of the Sea and in national dealing between developed and developing coastal states.

Let us take these three basic requirements and look at them more closely. (1) The second half of the twentieth century is the era of 'development'. The United Nations Development Programme has been established and many developed countries have set up development agencies to provide aid to developing countries. Such agencies usually

work through bilateral agreements, sometimes using the UN specialised agencies in the process. At the same time, the developing countries are clamouring for what is known as 'transfer of technology', as if technology consists of something that can be handed over in the form of a package bringing immediate affluence to the recipient. In fact, much of the money poured into 'development' over the years has been wasted due to two basic factors: first, lack of basic research before the actual development project is started; and second, inadequate training of personnel in the developing country to enable the project to be undertaken in the first place using local skilled personnel and, even more important, to ensure that the activity whatever it may be, continues in full operation when the basic project is complete and external support, both in the way of funds and personnel, is withdrawn.

This is just as applicable in the field of marine science, and marine affairs generally, in which the real lack of most developing countries is not technology as such and frequently (particularly in recent years) not even funds, but trained personnel and a viable infrastructure bringing together all the various interests—universities, navy, fisheries, basic research interests, marine engineering and so on.

Once an educational system has been devised that will produce a regular supply of trained personnel on the market, a country is able to absorb outside technological know-how and adapt it to its national needs. In the field of marine science, the best example of a developing country whose administrators and decision makers have realised this truth, is Mexico which is well on the way to becoming self-sufficient in marine scientific manpower and therefore a very powerful nation in marine affairs generally.

There is a real need to educate scientific administrators and decision makers in developing countries to the fact that there is no short cut to the wealth of the ocean's resources and that their highest priority should be the development of a cadre of trained manpower.

(2) There is no doubt that coastal-state control over scientific research in the economic zone is here to stay but there are far too many senior scientists unable to assimilate the fundamental political changes that have taken place in their chosen area of activity. Fighting the inevitable can do more harm than good as it can only strengthen the belief, sincerely held in many countries with no fundamental scientific background, that scientific research in the oceans is a cover up for other activities which would be to their detriment.

In the past, senior scientists planning research cruises have as a concession offered a few places aboard ship during a cruise to one or two scientists from the coastal states off which they wish to operate. Developing country scientists that have participated in such cruises have almost unanimously declared that they have been valueless both to themselves and to their country, as they have come unprepared to a sophisticated activity run by a research group having close personal relations and a full knowledge of the work being conducted; as a result, they have found themselves as outsiders with little understanding of what is going on.

In the future it must be recognised that the research is being conducted in what is virtually the national territory of the coastal state, just as if it were taking place on that part of the country which is above water. As a result the coastal state, through its marine scientists, now has the full right to participation in the research project from start to finish. This means that the coastal state scientists must be fully involved in the project from the first planning stages, through the data collection/cruise phase, to the data analysis phase and the assessment and writing up of the results. Should it be more convenient,

due to availability of facilities, development of equipment, and so on, to carry out the first and last phases in a laboratory in a developed country, for example of, that providing the research vessel, this is where the developing country scientists will have to work at full cost to the developed country. In fact the trend should be towards developed country scientists setting up facilities in developing country laboratories (at developed country expense), in order to help developing countries to strengthen their own facilities.

Whilst the developed country scientists have got to recognise the essential change in their working conditions if their science is not to die, the scientific funding bodies and aid agencies must come to realise that they are in this together and that this is a joint activity within the mandate of both bodies. The first reaction of a well known funding agency to such a suggestion was "we cannot afford it" and no doubt initially aid agencies would have a similar reaction against what they would consider to be funding national research projects, even if in the waters of countries they are set up to aid. Such attitudes must be modified drastically in the recognition that this is not only to the benefit of both in the long term but is basically the only solution if global marine science is to survive. A logical development would be to bring the two activities under the same administration and no doubt this will be the final outcome in many countries, but such moves will clearly only come about in slow time.

(3) Finally, there is the widely recognised need for basic education and training of young marine scientists in the developing countries (as touched upon in (1) above).

There are many difficulties inherent in the development of a viable infrastructure in a country with little or no tradition in the marine sciences, such as wastage and 'brain drain', which would be out of place if discussed in this article, but building up a strong cadre of marine scientists in each coastal state is the only way of overcoming the almost pathological aura of fear and suspicion that has grown up in many developing countries with regard to marine scientific research. It will only be when countries have their own trained scientists to whom the politicians can turn for advice, or, even more important, who can apply pressure on the politicians to take action, that this fear and suspicion will subside. The intergovernmental bodies will have a growing role to play in this regard if they can be so structured to gain the trust of the developing countries but they can never replace a strong national cadre of trained marine scientists.

It will be noted that education and training of marine scientists in developed countries has been omitted from this list. This is because it is considered to be a national problem for the developed countries concerned. It must however be clearly recognised, and soon, that education and training of marine scientists in developing countries is an international problem requiring urgent and greatly increased action.

Bright future?

I am convinced that the future for the marine sciences is bright but that this will depend on two basic factors: first, the end of the UN Conference on the Law of the Sea and a stable period during which the results and implications thereof can be assessed and put into practice; and second, the recognition and acceptance of a new and very different international regime for marine science in the future, by those who have been brought up to believe in their historical right to conduct marine scientific research where and when they please (subject only to finance). When these two factors have been met, such a regime could lead to accelerated progress in the marine sciences through a greatly enlarged global community of scientific colleagues working towards common scientific goals.

1967-77: Ten years of marine geology and geophysics

Xavier Le Pichon

CNEXO, B.P. 107, Paris 75116 and COB, B.P. 337, 29273 Brest, France

Plate tectonics gave a great stimulus to marine geology and geophysics; in the years since its enunciation there have been major drilling programmes, a new science of global palaeoenvironmental oceanography and much more detailed oceanographic surveys. The pace shows no sign of slackening—in the next few years the passive continental margins will be much studied.

In April 1967, Jason Morgan¹, having unexpectedly changed the subject of his talk, demonstrated to a large audience at the American Geophysical Union meeting, in Washington, how sea-floor spreading in the Atlantic Ocean had occurred between two rigid 'crustal blocks' moving apart on the surface of the spherical earth. This 'plate tectonics' model of the Earth's surface was implicit in the sea-floor spreading hypothesis of Hess², with its two corollaries of Vine and Matthews³ magnetic lineations and Tuzo Wilson⁴ transform faulting. But although the magnetic anomaly profiles across the East Pacific rise had convinced many earth scientists by early 1966 of the reality of sea-floor spreading, the demonstration made by Morgan was largely ignored by the audience. It is only during the following years that the full impact of rigid plate kinematics led to the present revolution in the Earth sciences. And this revolution has been most spectacular in marine geology and geophysics, which, with earthquake seismology, furnished most of the discoveries on which it is based.

It is in the field of global kinematics, past and present, that the progress has been most rapid. Because plates are rigid, it has been possible to construct a fairly precise global quantitative model for the present interaction of the 11-12 major plates, along their boundaries which are generally covered by several kilometers of water. In addition, it has been possible to decipher, at least in a preliminary way, the complex kinematic history of all large oceans, through the powerful Vine and Matthews method of dating the igneous oceanic crust. Thus, for the first time, there is a successful quantitative model of the dynamic evolution of the earth surface, a model which has been repeatedly tested, in particular through the use of deep-ocean drilling.

Deep-sea drilling

In 1968, at the time when the plate tectonics model was being accepted by the scientific community as the working model, the D. V. Glomar Challenger set out to drill her first hole through the floor of the Gulf of Mexico. She is now drilling her 418th hole and the results⁵ obtained by the Deep Sea Drilling Program (DSDP), under the sponsorship of JOIDES (Joint Oceanographic Institutions for Deep Earth Sampling) have shown that the proposed kinematic evolution of the oceans is supported by the analysis of the sedimentary column and the underlying igneous crust. In particular, the magnetic reversals absolute timescale, which is the basic tool to unravel the history of the oceans and which is now known from present to Middle Jurassic (165 Myr ago) with a relatively good precision (≈ 10 Myr), was entirely calibrated by deep-sea drilling results.

The single most important accomplishment of deep sea drilling, however, was the creation in the last 3-4 yr of a new field of science—global palaeoenvironmental oceanography. This occurred as it was progressively realised that the drastic changes in the Earth surface configuration are highly correlated to changes affecting the hydrosphere and the atmosphere, but also the biosphere. It now seems possible to obtain reasonable physical models which broadly account for the deterioration of climate during the last 40 Myr, simply by taking into account the different ocean-continent distribution pattern (for example, ref. 6).

Palaeoenvironmental oceanography

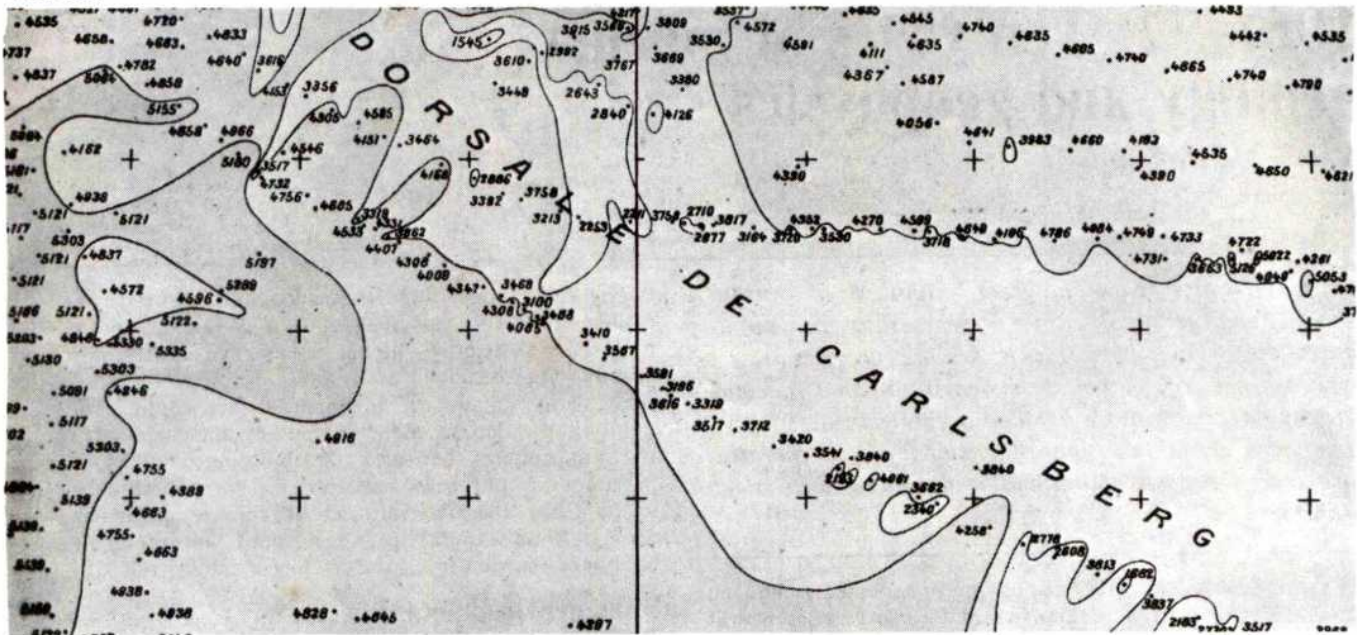
In the last 200 Myr the Earth surface configuration has changed from a supercontinent, Pangea, surrounded by a proto-Pacific super-ocean, Panthalassa, to the present fragmented ocean and dispersed continents. Panthalassa had a western prolongation, called Thetys, inserted like a wedge between Eurasia to the north, and Africa, India and Australia to the south. Its margin was almost entirely a consuming plate boundary where oceanic lithosphere was being subducted under the continent. Very little is known about its palaeo-oceanography as most, if not all, of the Triassic (~ 200 Myr) lithosphere has since returned into the mantle. Future drilling may, however, obtain Lower Jurassic sediments from the oldest portion of the Pacific ocean, which is now in the process of disappearing along the western consuming plate boundary, thus providing essential data on Panthalassa.

There is much better information on the evolution of the world ocean during Mesozoic, 165-60 Myr ago, which was characterised by ocean basins in an early stage of evolution, the newly born Atlantic and Indian basins, by a mature but shrinking proto-Pacific and a disappearing Thetys. The breaking up of Pangea resulted in the creation of rifted inactive margins where a great proportion of the Earth's sediments have now been trapped, bordering shallow (≈ 3 km) and narrow basins underlain by hot oceanic lithosphere. The ocean environment under an equable climate, was fairly tranquil with thermo-haline homogeneity and mostly latitudinal, probably wind-controlled circulation pattern (for example, ref. 7).

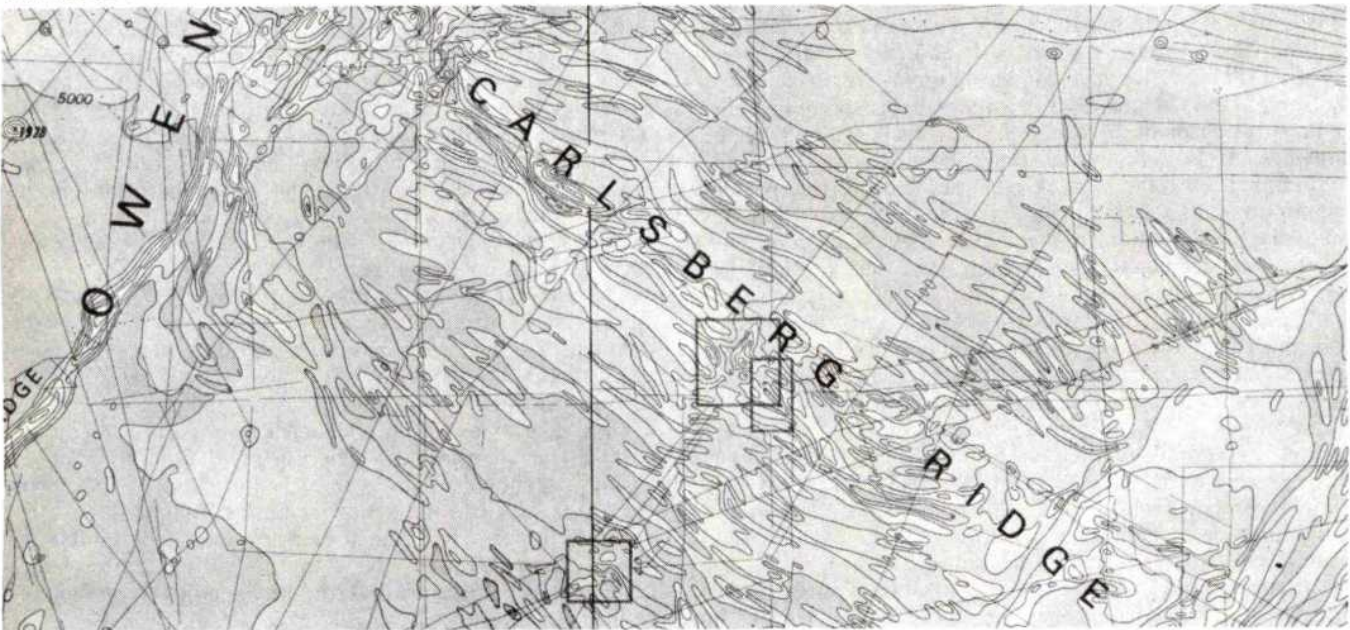
The new ocean basins continuously widened but also deepened, as they became older. The relationship between ocean-depth and age of the plate is a direct consequence of the lithospheric plate being a thermal boundary layer which moves away from its zone of creation at the axis of the mid-ocean ridge. It is a powerful tool to reconstruct the palaeobathymetry of a given ocean, knowing its age.

This age-depth relationship also implies that the average depth of the world ocean is related to its average age. Thus a faster rate of lithosphere renewal should lead to overflow over the continental borderlands, and vice versa. This effect may be the principal cause of the worldwide transgressions and regressions, outside of the geologically relatively recent changes in the volume of the ice-caps. The well known Upper Cretaceous (80 Myr ago) transgression, during which 85% of the Earth was covered by water (compared with 71% today and 65% during the largest regressions), has been explained in this way⁸. In any case,

Then.....



.....and now



A General Bathymetric Chart of the Oceans was first published in 1903 by Prince Albert of Monaco. The upper picture shows a portion of the 1938 edition for the Indian Ocean just east of Somalia (1,400 × 900 km). This chart was still in use at the beginning of the International Indian Ocean Expedition in 1959. The 1975 edition (lower) shows the same area as now charted. The Carlsberg Ridge is now seen to run into the Owen Fracture Zone. Contours are at 1,000 m spacings; ship's tracks are marked lightly.

recent oceanographic, mostly oil-applied research, has demonstrated the existence on the continental margins of numerous global unconformities which are related to sea-level changes. It now seems possible to reconstruct a fairly precise history of the evolution of sea level during the last 200 Myr.

The radical change brought by the Cainozoic (<60 Myr) era, which transformed the warm ocean and mild climates

of the Mesozoic into the cold ocean and glacial climates of the Quaternary (<3 Myr) has been described as a 'commotion in the ocean'. The most dramatic change occurred 38 Myr ago with a drop of about 10 °C of the deep water temperature⁹. This was related to the formation of a circumantarctic seaway, with separation of Australia from Antarctica and the opening of the Drake passage, and to the closure of the Tethys. Strong thermo-haline deep

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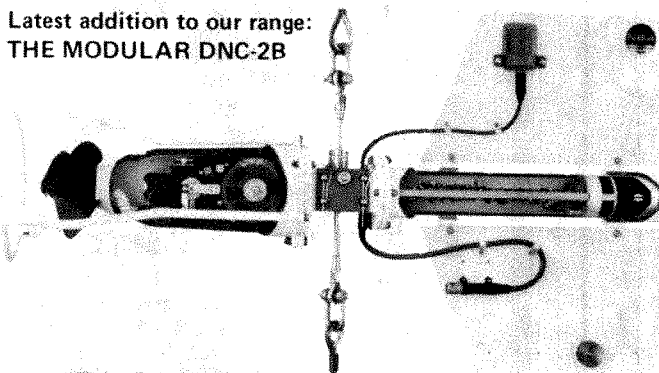
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circulation became well developed, resulting in widespread sea-floor erosion and the present meridional as well as latitudinal circulation pattern was established at this time.

But, it is still not understood why, whereas the first sea-ice appeared 38 Myr ago, the Antarctic ice-sheet did not develop for another 20 Myr. As for the Arctic ice-sheet, it started to develop 3 Myr ago and this event may be related to the creation of the Central America land bridge. The history of the repeated Late Pliocene–Pleistocene (<3 Myr) southward transgression of polar water by as much as 20° of latitude is now being very precisely reconstructed. One can expect in the years to come major progress in the understanding of this Cainozoic ‘commotion’ from such ocean sediment studies and from oceanologic and climatic modelling.

Geochemistry and geology

The role of the world ocean as a geochemical sink is also being progressively revealed. The degree of oxygenation of the deep ocean has greatly changed. It seems to have been mostly poorly oxygenated during Mesozoic whereas it is highly oxygenated nowadays. These changes probably deeply affected the carbon cycle in the hydrosphere and the carbon dioxide contents of the atmosphere. The development of calcareous and siliceous microorganisms during the Mesozoic must also have introduced a basic change in this geochemical complex cycle.

In contrast, there has been relatively little progress in our understanding of the geological structures generated or modified by the processes of plate tectonics, and first the oceanic lithosphere itself. A striking example concerns its upper portion: the oceanic crust. We know its age nearly everywhere but we still do not know unambiguously its structure and composition. Fifteen years ago, marine geophysicists had proposed, on the basis of seismic refraction results, that the oceanic crust had a homogeneous and uniform three-layer stratification (1, sediments; 2, basement; 3, ‘oceanic layer’) on top of the upper mantle. Layer 2 was supposed to be made of basalt and layer 3 of gabbro. Hess later proposed that layer 3 consisted instead of serpentinised peridotite. Today, the controversy is still not resolved. The oceanic crust models proposed nowadays are partly based on the results of scattered, poorly located dredgings out of a precise structural frame work but they mostly draw on analogies with ophiolites and predictions based on highly simplified theoretical models.

To this day, drilling has failed to shed much light on this problem because of technological limitations. The largest penetration in the igneous crust does not exceed 600 m,

that is about one tenth of the thickness of the crust. In addition, the necessity to dispose of a least 100 m of sediments over the igneous crust to stabilise the drill bit excludes most of the actively accreting boundaries from being sampled. Consequently, no direct information can be obtained on the actual processes of formation of the crust by drilling. It is fair to conclude that our poor knowledge of the oceanic crust results from the scarcity of direct geological evidence.

An important development, in this domain, has been the introduction of a much more refined seismic refraction investigation of the ocean crust and lithosphere, with ocean bottom seismographs (the so-called OBS) and better inversion techniques. The resulting acoustic structure is apparently quite complex and variable, as would be expected. Layer 2 seems to be a zone of strong velocity gradient with an uppermost very low velocity zone which progressively disappears with age. There is no generally accepted standard structure for layer 3 and its evolution with age, although it has recently been proposed that a rapid thickening away from the rise crest is due to the formation of a low velocity zone at its base. There is some fairly strong evidence for the existence of a magma chamber at a depth of 2 km below the sea floor under the East Pacific rise¹⁰. No such magma chamber could be detected under the crest of the slower spreading mid-Atlantic ridge.

Very long seismic refraction lines and earthquake seismological studies (primarily surface waves) have given some important information on the structure and evolution of the lithosphere with age. If the thickness of the lithosphere is defined by the lid of the underlying low velocity zone, there is a definite thickening with age from less than 50 km to 80–90 km. In the accretion zone, under the crest of the mid-ocean ridge, where the lithosphere is being created, a zone of partial melting comes within a small distance from the sea floor. Strong internal stratification seems to be common within the lithosphere.

Although this whole field of direct modern investigation of the structure and evolution of the crust and lithosphere by explosion and earthquake seismology is very promising, it is still in its infancy and it is too early to make firm conclusions. Note that an interesting new technique may well be the use of commercial multi-channel seismic reflection for recording oblique reflections from the crust and mantle. Another important new technique will certainly be the use of seismic receivers in the deep-sea drilled holes.

In the absence of detailed direct measurements, physical models based on plate tectonic concepts could bridge the

Mechanical arm of CNEXO submersible CYANA sampling a piece of pillow at 2,700 m depth in Transform Fault A in the Atlantic Ocean during the FAMOUS project. Part of a vertical dyke is seen in the background and numerous signs of animal life.



gap in our knowledge. It is true that it is relatively easy to explain heat flow and water depth variation as a function of age (see ref. 11, for example). But, this success does not mean that the formation of the oceanic crust and lithosphere is understood. Most of the proposed models are purely thermal and ignore the mechanical definition of the lithosphere. Further, widely different sets of parameters can be chosen, provided that the principle of a rigid thermal boundary layer is preserved. And, most important, their results away from the axis do not depend on the mode of transfer of material from the asthenosphere to the lithosphere in the rather narrow accreting zone. Yet, the knowledge of this mode of transfer is essential to any modelling of the formation of the lithosphere and crust. Clearly, additional constraints are needed. An interesting new one has recently been shown to be that the ocean depth does not increase as fast beyond 60–80 Myr as would be required if it were the cooling of a thermal boundary layer, which indicates the existence of additional sources of heat.

Thus, our knowledge of the ocean crust and lithosphere structure is still schematic. Is there a clearer understanding of the geological structures created by the interaction of plates along their boundaries? In fact, most of the geological models proposed for a consuming plate boundary (deep-sea trench), for an accreting plate boundary (spreading centre), or for a transform boundary are in great part hypothetical and grossly simplified.

Yet, approximately 120,000 km of length of plate boundaries lie below 2.5–11 km of water, which have no equivalent or directly comparable tectonic features above sea level. This is about 80% of the total length of the boundaries of the well defined plates. It is along the oceanic accreting boundaries that the tectonic fabric of the ocean floor is created. It is along the oceanic consuming boundaries that some of the tectonic fabric of the future subaerial mountain belts is presumably set into place. An understanding of the process of formation of the oceanic crust requires knowledge of the spatial and temporal distribution of the volcanic and tectonic events leading in turn to a full comprehension of the pattern of geochemical variation within the accreting plate boundary area. An understanding of the process of subduction also requires a knowledge of the spatial and temporal distribution of the tectonic features within the consuming plate boundary area and this, in turn, can lead to important information on the rheology of the plate itself. Clearly, any coherent development of the plate tectonic model passes by an adequate study of these boundaries.

FAMOUS

Standard oceanographic techniques currently used by most investigators are not adequate for this type of detailed geological and geophysical surveys as they have a spatial resolution which is not much better than half of the water depth. As a result, so-called detailed surveys often have a grid spacing of 10 km or more. Yet, studies of magnetic anomalies using precisely navigated deep-tow studies, have shown that the width of the zone of creation of oceanic crust in accreting plate boundaries is 2–6 km. To resolve processes occurring on such a scale, one needs to use techniques having a much better resolution with very precise navigation. These techniques should of course include bathymetry, seismic refraction survey and detailed geological surface mapping with precisely located multiple drillings. Such a complete study has not yet been performed. The FAMOUS program has made an important step in this direction^{12–14}, however.

The French American Mid-Ocean Undersea Study (FAMOUS) was designed 'to conduct an integrated and detailed geological, geophysical and geochemical study of a portion of plate boundary where new oceanic crust is generated' in the North mid-Atlantic ridge. The main

characteristic of this study is that, for the first time in deep ocean, object having a size from a few centimetres to kilometres were mapped and placed within the framework of a precise structural context. This was done in a first phase with the help of precisely navigated surface ship studies using the powerful multi-narrow beam echo-sounder, deep-tow fish and transponder located dredges and bottom photographs. Then, in a second phase, precisely navigated manned research submersibles established well documented geological sections, with both tectonic observations and rock sampling. Thus, methods of classical field geology could be applied to the deep ocean floor. As a result, a precise structural and petrological model of the Rift Valley and intersecting transform fault has been obtained for the first time.

But FAMOUS was just a beginning. There is little doubt that future research will rely more and more on studies with the same amount of resolving power, from seismic refraction to field geology. A good example is the problem of hydrothermal circulation in newly formed oceanic crust which has been shown to have a major role in the cooling of the crust, the distribution of heat flow, the formation of mineral deposits leached from the crust and the general geochemical cycle of seawater. The vents through which hot water comes to the sea-floor have such a small size that they require high-resolution techniques to be studied. The research submersible Alvin is now making such a study on the Galapagos rise, in the Eastern Equatorial Pacific ocean.

Perhaps, because of the formidable depths at which they lie, consuming plate boundaries have been studied in less detail¹⁵. However, a great step forward in their exploration was made with the acquisition of good commercial seismic reflexion lines revealing the structure of the inner wall which actually consists of a sedimentary accretionary wedge, made of imbricated thrust sheets which is several tens of kilometres long and up to tens of kilometres thick. The actual process of formation of this complex wedge is still not well understood and may include alternating phases of erosion and accretion. It has even been proposed that considerable erosion of continental crust may occur. The importance of such processes in an understanding of the geodynamics cannot be over emphasised.

The future

The next 2 yr, which will see the first systematic attempts at drilling consuming plate boundaries, should shed considerable light on these problems. But a much greater effort is needed to resolve their detailed structure and tectonics. For example, the detailed pattern of microseismicity across a trench is still essentially unknown, although a few OBS measurements have been made.

It is quite probable, however, that, in the next 4–5 yr, the greatest effort of the marine geoscience community will go towards solving the structure and evolution of passive continental margins which contain, as mentioned above, up to 50% of all the Earth's sediments. In addition to their potential economic interest, the passive continental margins are probably the only places where one can find the record of the early stages in the formation of a new ocean. There is still considerable disagreement on such fundamental problems as the nature of the continental crust—ocean crust transition, the actual boundary of the original continent, the processes which have allowed the thinning of the continental crust and the relative importance of the possible processes of subsidence. The ultimate tool for this type of investigation, very deep drilling, is actually being actively considered in spite of the very large costs involved.

For this review to have been truly complete, many other results as well as planned investigations would have had to be mentioned. In addition, no single writer can possibly cover adequately such a large field, so this review is certainly somewhat biased. There is, however, little doubt that the large scale exploration phase of the world ocean

made in the framework of plate tectonics in the last 10 yr is now coming to an end. The next phase will require much more elaborate, more costly techniques, having a greater resolving power to lead to a correct modelling of the various physical and chemical processes involved in the functioning of the great lithosphere engine.

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Breaking waves

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Why do waves break? What happens during breaking itself? More than a hundred years of mathematical studies still leave some aspects of these questions unanswered, although we are now understanding much about the processes leading up to breaking and the period after breaking has occurred.

BREAKING waves, both whitecaps and surf, are commonly observed in the ocean, but surprisingly little is known about them. Yet they are important for a variety of reasons. They place large hydrodynamic loads on man-made structures, transfer horizontal momentum to surface currents, provide a source of turbulent energy to mix the upper layers of the ocean, move sediment in shallow water, and enhance the air-sea exchange of gases and particulate matter. To further our understanding of these processes we must first understand the dynamics of wave breaking.

The scientific study of breaking waves is difficult for a variety of reasons. In theoretical investigations the equations of motion are nonlinear, and the boundary conditions (also nonlinear) must be applied at the free surface whose location is not known beforehand but must be determined as part of the solution. Observations are hindered because breaking occurs rapidly and intermittently. In the ocean large wave forces tend to carry away the instruments; whereas measurements made in controlled laboratory conditions cannot always be scaled-up owing to the relatively greater importance of viscosity and surface tension at the small scale.

At the present stage of theoretical development simplifying assumptions are commonly made about the flow-field in a wave. The dynamics of ocean waves are dominated by the inertia of the fluid, the pressure gradient, and the acceleration of gravity. For large scale waves the fluid viscosity can be neglected in the bulk of the fluid since it is important only in very thin layers near the fluid boundaries. Similarly, surface tension usually can be neglected unless the free surface is very highly curved. It is mathematically convenient to assume the fluid to be irrotational, that is, to have no vorticity—a measure of the rotation which an individual element of fluid has about its centre. This will be true if a wave propagates into a region of inviscid fluid previously at rest. It will always be a very good approximation unless there is a strong current shear or the flow becomes turbulent. In the latter case the fluid can still be irrotational outside the turbulent zone. The mathematical simplification is considerable since in an irrotational wave the vector velocity may be expressed as the gradient of a scalar (the velocity potential function) for which a linear equation (Laplace's equation) holds. We can neglect the airflow above the water since the density of air is 10^{-3} times that of water. Finally it is useful to consider the flow to be two-dimensional, that is, to neglect variations in a direction parallel to the wave crest. As more is

learned about the wave motion the effects of these neglected quantities can be incorporated.

It will be helpful to review some of the properties of simple waves which do not break. A wave is characterised by its wavelength (λ), water depth (d) and wave height (H , see Fig. 1). It is not the absolute values of these quantities but their ratios which are important. When the water depth is greater than about half of a wavelength the wave is in deep water, and it does not feel the effects of the bottom. Smaller values of d/λ correspond to shallow-water waves, and in the limit when d/λ approaches 0 a solitary wave exists, so-called because it consists of a solitary crest moving in shallow water with troughs infinitely far away. Many of the simple properties of non-breaking ocean waves can be explained in terms of linear, small-amplitude theory which is an approximation to the complete theory of wave motion valid when the wave steepness (H/λ) and height-to-depth ratio (H/d) are much less than 1. In deep water when a small-amplitude wave passes, individual particles of water do not travel along with the wave, but instead they move in closed circular orbits whose radii decrease exponentially with depth as shown in Fig. 1. In shallow water these trajectories become ellipses with shortened vertical axes, and for a solitary wave each fluid particle moves forward with a hop as the wave passes. The profile of a small-amplitude wave is symmetric about the vertical line passing through its crest.

Research work on breaking waves can be divided into three categories: those concerning waves (1) before, (2) during, and (3) after breaking. In this review we shall concentrate on some of the latest results in the first two categories.

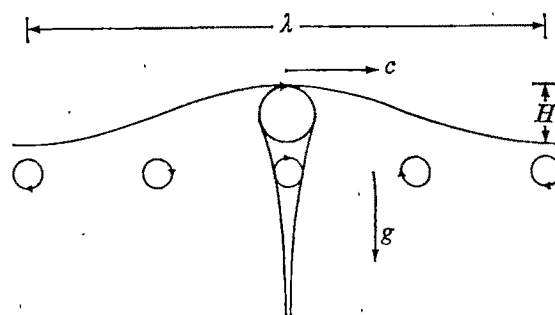


Fig. 1 The profile of a small-amplitude, sinusoidal wave in deep water showing the circular orbits of fluid particles with arrowheads indicating the direction of water movement at various locations. The rapid decrease of the orbital radii with depth is illustrated beneath the crest. The wave of wavelength λ (the horizontal distance between successive troughs or crests) and height H (the vertical crest-to-trough distance) is moving to the right at speed c with gravity g providing the external restoring force. (This wave is too high relative to its wavelength for small-amplitude theory to hold, but it was drawn this way for purposes of illustration).

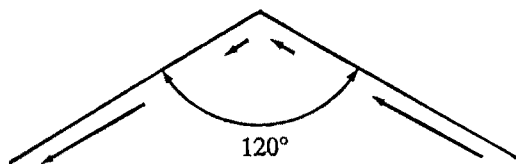


Fig. 2 A close-up view of the crest of the highest, steady wave as seen from a reference frame moving with the wave to the right. Fluid particles come to rest at the sharp wave crest.

Before breaking

One approach to the study of breaking waves has been to start with a small-amplitude wave and to consider progressively higher symmetric waves which propagate without change of shape in water of constant depth. For a fixed wavelength and depth there exists a highest wave which is just on the verge of breaking. When viewed from a reference frame moving with the wave the flow is steady, that is, independent of time, and this simplifies the problem. Analytically it is possible to begin with the small-amplitude approximation to the fluid motion and to correct this for the effects of increased wave height. This is then recorrected and so on until sufficient accuracy is obtained. Each successive correction may be thought of as one member in an ordered series of corrections called a perturbation series. The importance of any correction relative to the one before it is measured by a perturbation parameter which is related to the wave height. To get an accurate answer for higher waves the perturbation series must be taken to higher order, that is, more and more corrections are needed. Sir George Stokes found the third-order correction in 1847 (ref. 1) for periodic waves in water of arbitrary, uniform depth and the fifth-order, deep-water correction in 1880 (ref. 2). His results indicate that for low-but-finite-amplitude waves the speed of wave propagation (the phase speed) increases with the wave height. The fluid particles no longer move in closed orbits, but they acquire a small, added, down-wave drift called the Stokes drift. Also the wave profile does not remain symmetric about its mean level as in a small-amplitude sinusoidal wave, but instead the crests become higher and sharper and the troughs flatter. Unfortunately it is not possible to carry out the Stokes expansion to a high enough order by hand to accurately determine the properties of the highest wave because the amount of algebraic manipulation required increases dramatically with order. Indeed the tenth-order, deep-water correction was not obtained until 1914 (ref. 3), and the fifth-order, general-depth one was found in 1955 (ref. 4). Slow progress has also been made for solitary waves⁵⁻¹² with the third-order result being obtained in 1971 (ref. 12).

A separate and completely independent line of reasoning yields some information about the highest wave. In general at a wave crest the fluid particles are moving forward at a speed less than the phase speed (c), and as higher waves are considered the particle velocity approaches the phase speed. In a limiting wave these two velocities are equal. A further increase in wave height will cause the fluid particles to overtake the wave itself, and breaking will ensue. In the reference frame moving with the wave the water comes to rest at the crest, and it must attain a sharp point¹³ with an included angle of 120° as shown in Fig. 2. This corner-flow is only a first approximation valid very close to the crest and includes no information about the rest of the wave or about lower waves.

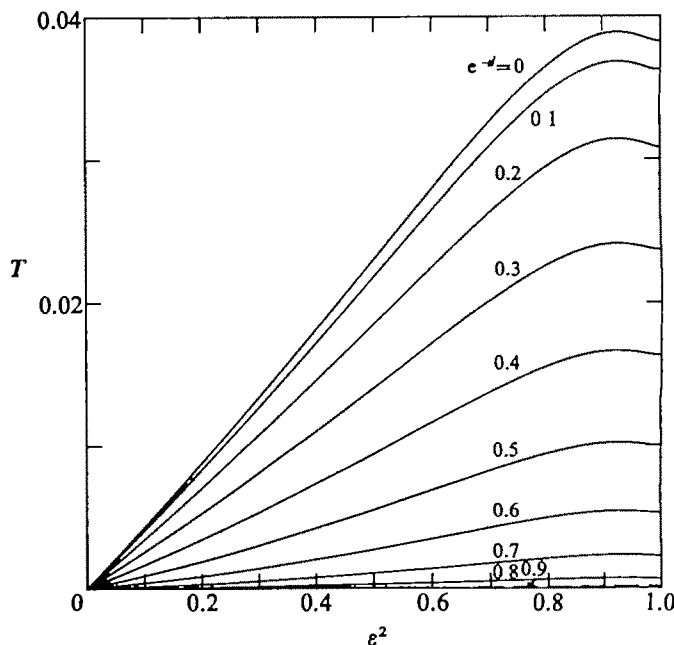
There have been several attempts to bridge the gap between the finite-amplitude theories and the highest wave. Some theoreticians have tried to build the 120° angle into the theories¹⁴⁻¹⁷, but it is not clear how this should be done¹⁸⁻²⁰. Many purely numerical approaches have been tried²¹⁻³⁰, and these are successful for small to moderately-high waves. They usually involve guessing the wave shape and then adjusting this until some error criterion is satisfied at a number of points along the wave profile. The advantage of these methods is that a high-

speed computer can be used to do the calculations, but often they fail to reach acceptable accuracy levels when the highest wave is approached.

Some recent advances have been made with perturbation expansion methods in which the laborious algebraic manipulations are carried out automatically by computer^{19,31-34}. The series can be expanded to a very high order (past order 100 in some cases) and summed efficiently thus providing accurate answers up to very near the highest wave. Stokes' original expansion procedure fails for high waves since his expansion parameter does not always increase with the wave height¹⁹; therefore two distinct waves exist for the same value of this parameter. The wave height itself can be used as an expansion parameter¹⁹, but its maximum value is not known beforehand. This difficulty is overcome by defining a new parameter (ϵ) in terms of the fluid speeds at the wave crest (q_{crest}) and trough (q_{trough}) in the moving reference frame³²⁻³⁴. The complete range of this parameter ($\epsilon^2 = 1 - q_{\text{crest}}^2 q_{\text{trough}}^2 / c^4$) is known from the beginning. For a small-amplitude wave the water streams past to the left at the phase speed ($q_{\text{crest}} = q_{\text{trough}} = c$, $\epsilon = 0$), and for a highest wave it comes to rest at the crest ($q_{\text{crest}} = 0$, $\epsilon = 1$). The wave height increases steadily with ϵ to values of $H/\lambda \approx 1/7$ for deep-water waves and $H/d \approx 5/6$ for solitary waves, but other wave properties behave in a surprising manner. The phase speed, momentum and energy increase with wave height initially, reach maxima, and then decrease as the highest wave is approached³²⁻³⁴. Therefore the highest wave is not the most energetic as had always been assumed. This behaviour is illustrated in Fig. 3 which is a typical plot of the kinetic energy of the wave against ϵ^2 for various ratios of water depth to wavelength³⁴.

The energy maxima can be explained in terms of the wave profile^{32,34,35}. At the crest the profile of the highest wave is above that of a slightly lower wave, but because of the sharp peak it falls away more rapidly and soon dips below the lower wave. Considered as an average over a wavelength, the highest wave is actually lower than an almost-highest wave; hence

Fig. 3 The average kinetic energy per unit surface area (T) of a steady wave plotted against the expansion parameter, $\epsilon^2 = 1 - q_{\text{crest}}^2 q_{\text{trough}}^2 / c^4$, for various water depths (d) (ref. 34). For each depth the highest wave corresponds to $\epsilon^2 = 1$. Notice that each curve reaches maximum energy for waves lower than the highest. Quantities are measured in units of density (ρ), wave-number ($k = 2\pi/\lambda$) and gravity (g).



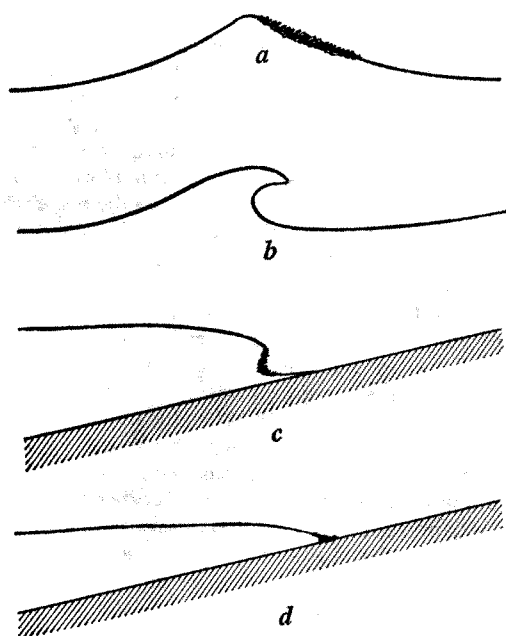


Fig. 4 The four main breaker types: *a*, spilling; *b* plunging; *c*, collapsing, and *d*, surging.

it has less energy. These surprising results have been confirmed by separate numerical³⁵ and analytical³⁶ approaches which are completely independent of the perturbation series.

The existence of the maxima in the wave property curves may have a bearing on why high symmetric waves begin to break^{32,34}. Usually a wave is specified uniquely by the water depth, wavelength and one other wave property. But because the curves of these properties bend over for very high waves two distinct states are possible with the same wave energy, say, but with different wave heights. The solutions corresponding to one part of the curve may be unstable, and a wave might jump from one part to the other. Alternatively symmetric waves may become unsymmetric at the peak of their energy curves making the highest wave inaccessible except through the loss of energy. Other possibilities exist, and there is scope for further research in this area.

Several laboratory experiments have been carried out to test the validity of the steady wave theories. Usually low waves are generated in a long, narrow tank with a paddle, piston or wedge-shaped plunger and are sent into a channel with a sloping bottom or converging side walls to build up their height. In the test section simple measurements of the water depth, wave height and phase speed can be made. The measured phase speeds and limiting wave heights agree with theory to within experimental error (a few per cent), but the observations are not precise enough to detect the phase speed maxima³⁷⁻⁴¹. Indeed it is difficult to produce very-high waves which remain steady and do not break. Whether this is due to the method of wave generation or due to an inherent instability has yet to be determined. In more complex experiments the surface profile, velocity distribution, particle orbits and accelerations have been measured⁴¹⁻⁴⁵. Comparisons with low-order, finite-amplitude theories (Stokes' third order) in deep water are acceptable, but they worsen as H/λ and H/d increase. Differences of 50% between the theoretical and the measured (theoretical > measured) fluid velocities are common for $d/\lambda = 1/10$ and wave heights of about half the maximum. This is unacceptable for design purposes since the hydrodynamic drag on a structure varies as the square of the velocity so that a 50% velocity error produces a 100% drag error. The most widely used numerical method²³ does somewhat better, but it is inaccurate for high waves in all water depths³⁴. The discrepancy between theory and observation is probably due to the low order of the theories used. Their slow convergence rate for

shallow-water is well known^{1,2}, and it is quite likely that the inclusion of higher-order terms will bring about a marked improvement. The results of the recent high-order calculations^{19,31-34} have yet to be compared in detail with experiments.

Surface tension becomes increasingly important as the curvature of the wave crest increases, and this effect will be amplified in a small-scale laboratory experiment where the wavelength is 10^{-2} that of a typical ocean wave. Surface tension makes it impossible for the highest wave crest to attain a sharp point, and what this will do to the energy maxima is not known. Ripples are generated at the highly curved crest, and these ride along on the forward face of the wave^{46,47}. At present no high-order theories include both gravity and surface tension, but this is a future goal. An exact theory does exist for pure capillary waves in which surface tension provides the restoring force and gravity is neglected⁴⁸⁻⁴⁹, but owing to viscosity this is hardly, if ever, applicable.

During breaking

We now turn to the time-dependent behaviour of waves as they steepen and overturn. Roughly speaking a wave will tend to break when the local energy density (E) exceeds a critical value (E_{max}), that of the most energetic steady wave of the same wavelength and water depth. This happens during shoaling when the energy scale (E_{max}) decreases shoreward, and E/E_{max} increases until the break point.

Results from laboratory experiments with shoaling waves indicate that breakers can be classified into four main types⁵⁰⁻⁵⁵—spilling, plunging, collapsing and surging—as illustrated in Fig. 4. The breaker type depends upon both the initial wave energy, characterised by the offshore wave steepness (H_0/λ_0), and the rate of energy input due to shoaling, characterised by the beach slope (s) (refs 40, 52-54, 56). A breaker parameter (B) representing the ratio of these two effects can be defined by $B = H_0/(\lambda_0 s^2)$ (refs 53, 54). As B decreases from large values there is a progression from spilling to surging breakers. A spilling breaker which occurs when a steep wave approaches a gradually shoaling beach is similar in profile to a steep, symmetric wave, but it has a turbulent plume of frothy water running down its forward face (Fig. 5). Apart from this turbulent region the flow is irrotational. A value of B of approximately 5 marks the transition from spilling to plunging. In a plunging breaker the wave steepens and overturns ejecting a jet of water from near its crest which eventually strikes the surface below (Fig. 6). The flow may remain laminar (non-turbulent) until right up to the point of impact, or the jet may become turbulent due to the growth of small scale instabilities induced by the wind and surface tension. The third breaker type is the collapsing breaker in which the wave overturns not at its crest but lower down the forward face, and a region of white water

Fig. 5 A spilling breaker in shallow water (photo copyright Institute of Oceanographic Sciences).





Fig. 6 A plunging breaker in shallow water (photo copyright J. D. Humphery).

forms probably due to the close proximity of the bottom. This type is not as clearly defined as the others, and it denotes the transition between plunging and surging at $B \approx 0.1$. The surging breaker moves up a steep beach as the forward tip of a low wave. It resembles a standing wave formed by total reflection from a vertical wall, but a small amount of turbulence may be present at the beach face.

Two of the shallow-water breaker types—spilling (Fig. 7) and plunging—can occur in deep water. The bottom slope is no longer relevant since the bottom is far away and does not affect the dynamics. Deep-water waves can receive an input of energy when they overtake or collide with one another, meet an opposing current or receive a 'push' from the wind. In a laboratory wave tank the energy input can be provided by a converging side wall which acts in a way similar to a sloping beach. There is a transition from spilling to plunging as the initial wave steepness decreases and the channel convergence increases⁴¹. For gently spilling waves $H/\lambda \approx 1/7$ which confirms that a spilling breaker may be thought of as a just-breaking, highest, symmetric wave. Collapsing and surging breakers do not occur in deep water, but their counterparts are a mixture of progressive and standing waves. In the limit when two identical wave trains propagating in opposite directions collide there exist pure standing waves. The highest standing wave of periodically recurring permanent shape has $H/\lambda \approx 2/9$ (refs 57, 58). Not much is yet known about the breaking characteristics of standing waves or of mixed waves.

Experiments with breaking waves in shallow water have concentrated on how the ratio of wave height to water depth at the break point (H_b/d_b) and the ratio of breaking wave height to initial wave height (H_b/H_∞) vary with initial wave steepness and beach slope. Some empirical relationships based on scattered experimental data have been derived⁵⁹, but not a great deal of confidence can be placed in them. Generally for spilling breakers H_b/d_b is in the range 0.65–0.85 (refs 50, 52, 54, 60) which is approximately that value obtained from the highest solitary wave theory. For plunging breakers H_b/d_b increases, and values as large as 1.3 and 3.0 have been measured for periodic^{50,52} and solitary^{60,61} waves respectively, H_b/H_∞ equals about 1 for gently spilling waves which break almost immediately upon shoaling and increases to about 2 for plunging breakers^{50,52}. The behaviour as the ratio of wave steepness to beach slope tends to zero is not well defined. If the slope is small but finite and the initial wave steepness tends to zero an incoming wave splits up into a series of solitary-like waves (solitons) of different heights, and the highest breaks first as a spilling breaker⁵⁴. But if the initial wave slope is held fixed and the beach becomes a vertical wall (s_∞), an incoming wave will be totally reflected if low enough or break as a surging breaker.

A few measurements of the velocity distribution in spilling and plunging breakers have been made in the laboratory^{41,42,50,52,62,63} and the field⁶⁴. Generally with increasing profile asymmetry the rapid-forward-velocity region beneath the crest deepens, and the velocity vectors there become tilted upward as in accordance with the formation of a plunging jet. On sloping beaches the backwash of the previous wave augments the velocity asymmetry and helps to topple the wave^{50,52,64}. In the most precise experiment⁴¹ the crest velocity of a deep-water plunging breaker was 1.6 times the phase speed just after the onset of breaking.

No analytical theories adequately describe the time-dependent process of wave breaking. Theories exist for small-amplitude, shallow-water waves, but these are approximations valid only when the wave slopes are low or when the fluid acceleration is small compared with gravity^{65,66}. When the free surface steepens the fluid acceleration becomes comparable with gravity and cannot be neglected. An attempt has been made to study the effect of a small perturbation on the 120° corner-flow⁶⁷, but this theory is not appropriate since the solution contains undesirable mathematical singularities both before and after the instant of breaking.

Modern numerical techniques have had some success in describing breaking. The method most widely used by ocean engineers involves actually measuring the profile of a breaking wave and then fitting an irrotational flow to this by minimising the departure from constant atmospheric pressure along the surface²³. It has three major disadvantages: (1) the wave shape is assumed to be steady which is certainly not true during breaking, (2) the method cannot represent overturned waves, and (3) it cannot predict the evolution of a wave. It does give a rough estimate of the velocity field, and experimental comparisons indicate velocity errors of 10%–30% beneath spilling breakers at the break point⁶³.

A series of finite-difference numerical methods have been devised to deal with free-surface deformations. The two-dimensional fluid region is divided up into a number of small cells, and the velocity and pressure gradients are approximated by their differences across the cells. Marked fluid particles denote the free surface, and these are moved every time step by velocities interpolated from the cell boundaries. An early method⁶⁸ allowed the free surface to curl over, but an artificially large fluid viscosity had to be used to damp out unwanted 'wiggles' (numerical instability). A modified inviscid method⁶⁹ is more accurate and has been used successfully to calculate the run-up of a solitary wave on a steep beach. But it fails at the point of spilling when a solitary wave approaches a gradual beach because it cannot cope with an overturning surface⁷⁰. Similar methods which allow the cells to move with the fluid should do better, but available computer resources limit the minimum cell size so that the cells become too distorted for acceptable accuracy as the wave steepens⁷¹. Attempts to rezone the cells lead to instability at breaking⁷².

A different formulation has made it possible to follow waves as they overturn⁷³. One of the properties of irrotational flow theory is that the shape of the fluid boundary and the value of the velocity potential on that boundary uniquely determine the flow-field inside. Therefore only the evolution of the free surface need be considered, and all the computational effort can be focused there to accurately resolve its shape. At a given instant the tangential and normal velocities of the surface which are needed to follow its evolution can be found by numerical differentiation and by the solution of a linear integral equation. Initially computational particles are distributed on the free surface, and their trajectories are calculated in time steps⁷³. Figure 8a (Cokelet, unpublished work) is a computer-generated plot of the profile of a periodic, deep-water wave at various times as it steepens and overturns. The initial wave moving to the right begins as a sinusoid but with too much energy ($E = 5/3 E_{\max}$) to remain symmetric. Figure 8b illustrates a portion of the wave profile near the crest as seen by an observer moving at the speed of a small-amplitude wave. The profiles, plotted from

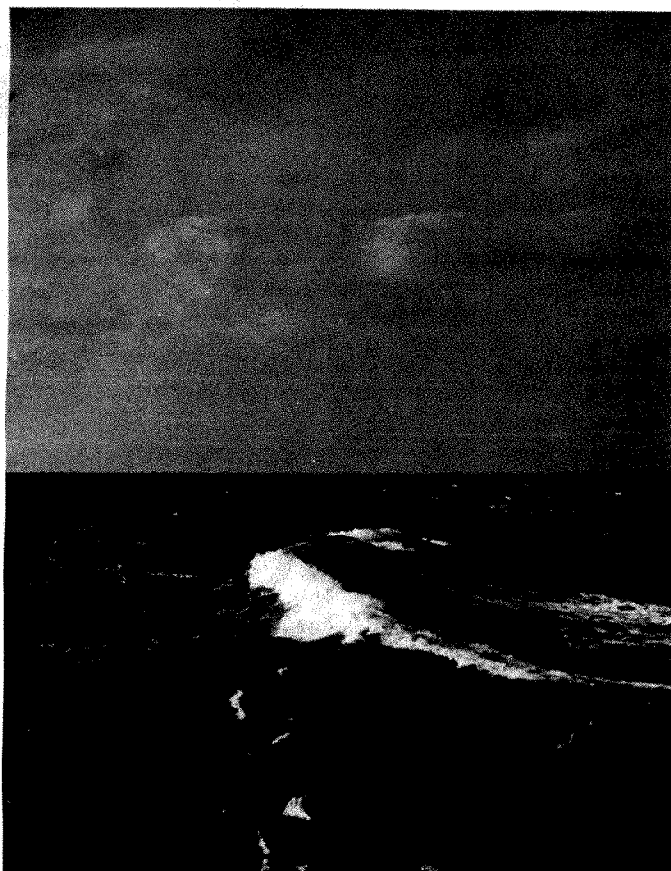


Fig. 7 A deep-water spilling breaker (or whitecap) moving toward the left (photo copyright J. R. Dunn).

the instant the wave face becomes vertical, show the crest toppling over towards the water below, but the theory does not remain valid past the point of impact. For spilling-like breakers the radius of curvature at the leading edge of the jet approaches the scale of the particle spacing, and the computations must be halted owing to numerical inaccuracies. Since irrotational flow is assumed there is no turbulent spilling, and the neglect of surface tension means no droplets pinch off. Numerical experiments are being conducted to determine how the plunging jet's momentum and energy depend on the initial conditions and energy input.

In some instances contrary to our simplifying assumption the airflow does have an effect on breaking. The wind creates a thin, sheared layer of water at the surface (the wind-drift layer), and this can augment the fluid particle velocity at a wave crest forcing the wave to break at a height below the maximum⁷⁴. Also the wind can blow-off or hold-up the wave crests, but this has not been studied. Conversely, breaking itself can influence the airflow, and this may help to explain how the wind makes waves. The airflow over a wave has a horizontally varying pressure field which must be shifted laterally with respect to the wave to feed energy into it⁷⁵. Theories which assume small-amplitude waves predict a wave growth rate which is much lower than that observed⁷⁵. But some new experimental findings show that breaking forces the airflow to separate from the wave surface thus shifting the pressure field so that it can do more work on the wave⁷⁶. Therefore wave growth is actually triggered by breaking which is a dissipative process.

After breaking

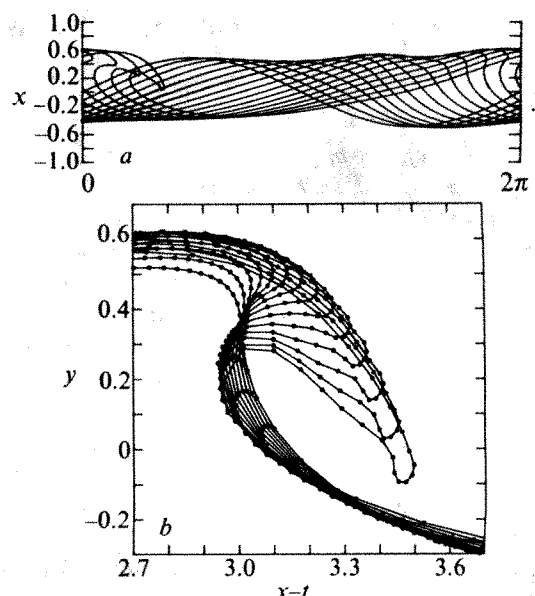
In shallow water after the period of rapid wave steepening and overturning there can exist a much less time-dependent period

in which the energy dissipated in the breaker approximately balances the increase of relative energy ($E/E_{\max}$) due to shoaling.

For a spilling breaker a turbulent, air-water mixture (a plume) runs down its forward face accelerated by gravity and retarded by the entrainment of up-slope momentum from the flow below. Theoretical considerations⁷⁷ based on the assumption of steady flow show that the plume can exist only if the surface slope and density difference between the whitecap and the water are sufficiently large. For a 30° surface slope corresponding to the 120° corner-flow, the plume must be about 8% air which is in agreement with observations of other self-aerated flows. The plume theory indicates that, once formed, a whitecap will tend to spread down the wave face, but energy balance considerations show that the ratio of whitecap length to wave height should be about constant⁷⁷. Since the wave height tends to diminish shoreward of the break point no true steady state can exist. It is observed^{55,77} that the whitecap grows, and its damping effect overcomes the effect of wave steepening due to shoaling. The wave crest becomes rounded which reduces the slope and allows part of the whitecap to be swept back over the crest. The wave then steepens, and the whole process repeats itself as long as sufficient wave energy remains⁷⁷.

After a wave has broken a step-like jump in the free surface can form and advance shoreward as a bore. Due to limitations of space we will not consider bores in detail but instead refer the reader to reviews on them⁷⁸ and on related hydraulic jumps⁷⁹. However, a recent numerical investigation which goes somewhat farther than previous ones should be pointed out⁸⁰. In this the run-up of a bore on a sloping beach is modelled. It is possible to follow the water up the beach until its forward tip gets very thin and then to follow its subsequent rush seaward (backwash) which itself develops into a bore. Preliminary results for a series of bores approaching periodically from the sea indicate that the maximum water velocity actually occurs during the backwash stage which may be of significance for sediment-transport studies.

Fig. 8 *a*, Computer-drawn profiles of a plunging breaker in deep water at successive time intervals of about $1/25$ of a wave period ($\Delta t = 1/4$ in units of p, k and g). The initial wave form is sinusoidal with its crest at $x = \pi/2$, but it has too much energy ($E = 5/3 E_{F_{\max}}$) to remain symmetric. Therefore the wave steepens and overturns. *b*, A portion of the crest plotted about every $1/50$ of a wave period ($\Delta t = 1/8$) as seen by an observer moving to the right with the speed of a small-amplitude wave ($c^2 = g/k$). The profiles are plotted from when the wave face becomes vertical, and the dots mark the computational particles (Cokelet, unpublished work).



For a tsunami the ratio of offshore wave steepness to beach slope is very small, and the wave usually does not break at all. In that case the maximum shoreward excursion of the water (the run-up distance) is of particular interest to those concerned with tsunami prediction and the design of shoreline structures. Advances have been made in the theory of wave run-up, but it is beyond the scope of this paper to consider them here (see ref. 81 for more details).

Conclusion

We understand some things about breaking waves especially before and after breaking when the flow is steady and more amenable to theoretical and experimental study. But there are large gaps in our knowledge. Much has yet to be learned about the time-dependent period during breaking. Laboratory experiments performed in shallow water have provided some information about this, and numerical investigations are likely to prove fruitful in the future. In this exciting field many questions remain unanswered. What are the velocity and acceleration fields in a breaking wave? How much of its momentum and energy are given up to ocean currents and turbulence? What is the spatial distribution and frequency of breaking? There is great potential for further theoretical and experimental research. The spectacular fury of a breaking wave stimulates the search.

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Acoustic oceanography

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Measurements of ocean finestructure (with typical time scales of 15 min to 1 d, spacial scales of 10 to 100 m vertically and 1 to 10 km horizontally) are consistent with the measured variability in the transmission of acoustic signals through the ocean volume. The observations suggest that the statistics of acoustic fluctuations can be used to monitor the statistics of ocean finestructure. Further, fluctuations in the travel time are a measure largely of temperature fluctuations along the acoustic path, and the difference in reciprocal travel times is a measure of the component, along the path, of current and current shear. Such acoustic measurements give integrals along the paths, with the attendant advantages and disadvantages as compared with the traditional 'spot' measurements; precision promises to be comparable or better.

OCEANOGRAPHERS differentiate between marine biology and biological oceanography. The former is concerned with biological processes in marine organisms; the latter places emphasis on biological processes in interaction with physical, chemical and geological processes. Biological measurements can serve as sensitive indicators of the source of water masses, of vertical motion and mixing processes, etc. It is in this sense that we distinguish acoustic oceanography from the intensively active field of marine acoustics.

During and directly following World War II, the interaction between the acoustic and oceanographic communities was close, to the benefit of both. Certain noises in the oceans could be traced to biologic origin, such as snapping shrimp. The deep scattering layer was discovered and readily associated with the known diurnal migration of copepods, towards shallow depths at dusk and downward at daybreak. (Details concerning the organisms among the migrating biomass that provide the

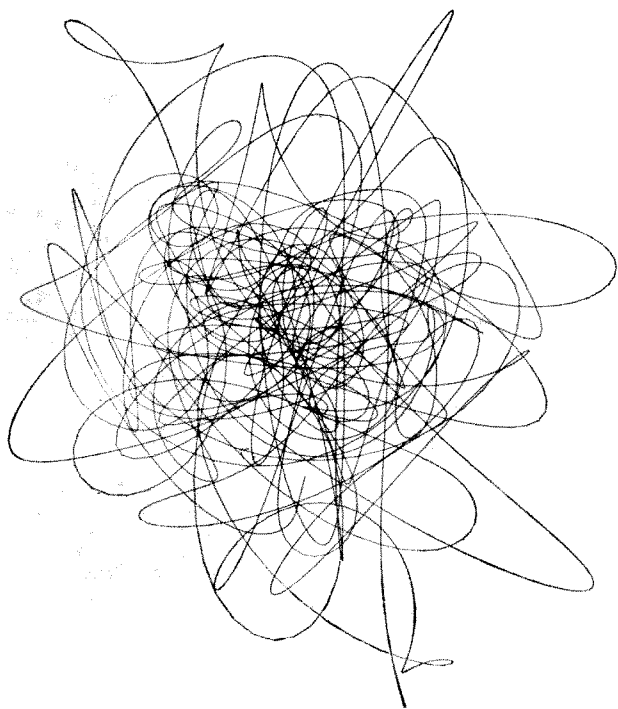


Fig. 1 Phasor diagram of acoustic transmission. The curve consists of straight-line segments through points at 5-min intervals. The distance of a point from the origin (not plotted) is proportional to the intensity, and its bearing angle to the phase of the received signal relative to that at the transmitter. The random walk character of the phasor diagram is associated with interference between many multipaths. The plot was made by computer simulation of the MIMI experimental conditions.

acoustic scattering cross section are complex and not totally solved even today.) The 'afternoon effect' of sharply reduced sonar ranges was explained quantitatively in terms of radiative warming of the near-surface waters. The existence of an acoustic wave guide (the SOFAR channel) was proposed on the basis of the known interior distribution of temperature and pressure, and dramatically demonstrated soon thereafter.

It was then generally accepted (primarily because the oceans are so transparent to sound and opaque to light) that sound propagation would develop into a primary tool for probing and monitoring the oceans. But by and large, the close cooperation between oceanographers and acousticians did not continue. We think this might have to do with the fact that the acoustic community became strongly concerned with the large variability in sound propagation; 20 dB fades are by no means uncommon. And here the oceanographic community was of no help. Oceanographers were preoccupied with very large scale, long time processes, d.c. oceanography so to say, that have little bearing on acoustic variability. What was needed here was a description of the ocean structure on a small scale, and the instruments for making the required measurements had not been developed.

And so the two communities went their own ways, to the detriment of both. The oceanographers were so preoccupied with their wind-spun oceans that they neglected the mesoscale and small-scale processes which contain (as we now know) 99% of the kinetic energy. The acousticians invented their own oceans, borrowing the concept of a homogeneous isotropic turbulence which had served as a successful model for explaining the fading of radio waves in the ionosphere. Temporal fluctuations were interpreted in terms of a frozen ocean structure drifting by (Taylor's hypothesis). But the ocean is neither homogeneous nor isotropic (except possibly on scales less than 1 m. Further, Taylor's hypothesis supposes that the mean currents (winds) exceed the intrinsic velocities of the structure. But in the oceans, the phase velocity of internal waves, for example, is typically 1 m s^{-1} , and the mean motion typically

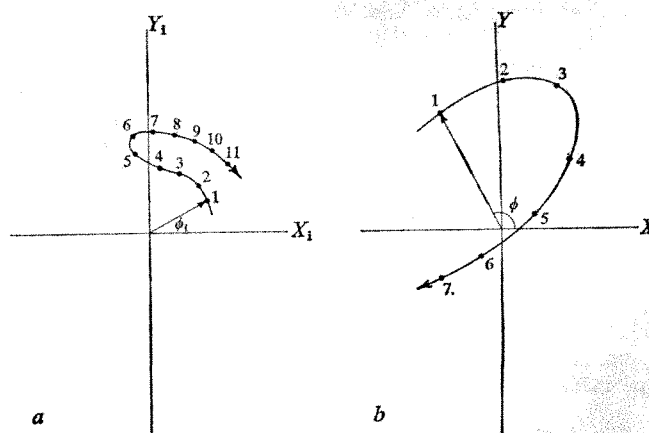
0.1 m s^{-1} . So there is no justification for Taylor's hypothesis, yet it remains ingrained in the acoustic literature (A. W. Ellinor has pointed out to us that most of the existing acoustic observations were carried out from moving vessels, and then the application of Taylor's hypothesis is entirely justified.)

In the 1950s it became apparent that discrepancies between successive measurements at repeatedly occupied oceanographic stations were not always the result of instrumental error. Since then, the development has been rapid. Ocean eddies with time-scales of several months and space scales of 100 km were found to be associated with 100 times the kinetic energy of the general circulation. At frequencies between a few cycles per hour (the Brunt-Väisälä frequency) and a cycle per pendulum day (the inertial frequency) internal waves dominate. Their vertical amplitude is remarkably large, 10 m in the upper oceans, up to 100 m at abyssal depths. The time was ripe to inquire whether internal waves could account for some of the measured variability in the propagation of sound through the ocean.

A long-range sound transmission experiment

In 1966 Steinberg and Birdsall conducted a pioneering sound transmission experiment across the Straits of Florida¹. Interpretation was made difficult by shallow-water effects, and subsequent efforts were shifted to a 1,250 km path from Eleuthera to Bermuda². An essential feature in these experiments (called MIMI for the Miami-Michigan participation) was that they gave continuous observations over many months, and this opened the way for a geophysical interpretation. In essence, the experiment consisted of transmitting a 406-Hz continuous wave signal, and of recording the relative phase and intensity of the received signal using a perfectly synchronised 406-Hz oscillator. The resulting time series of acoustic phase and intensity are dominated by occasional fade-outs and phase jumps which are the result of interference between the many paths from source to receiver (Fig. 1). This is an interesting problem in random-walk statistics, but unfortunately the ocean is involved only in a limited way: the determination of a single parameter, $\langle \phi_1^2 \rangle$, the mean-square rate of phase along any one single path (Fig. 2) (ref. 3). The parameter could in principle be measured directly if such a single path could be isolated from all other paths by a suitable directional antenna. But this is not possible, because each 'deterministic' path is split into 'micro-multipaths' (Fig. 3).

Fig. 2 Phasor diagrams for single path (a) and multipath (b) acoustic transmissions. The Cartesian coordinates designate the in-phase and quadrature components of the received pressure signal, at time 1, 2, ..., with the vector indicating the positions at time 1. ϕ_1 and $I_1 = 10 \log(x_1^2 + y_1^2)$ are the single path phase and dB intensities, and similar ϕ and I for the multipaths. Generally $\theta(t)$ varies more rapidly than $\phi_1(t)$, and $I(t)$ is more variable than $I_1(t)$. Passage of the vector trajectory near the origin (between times 5 and 6 in the right figure) is associated with a fade-out and a rapid change of phase by almost $\pm 180^\circ$, the sign depending on which side of the origin is passed (minus in the figure).



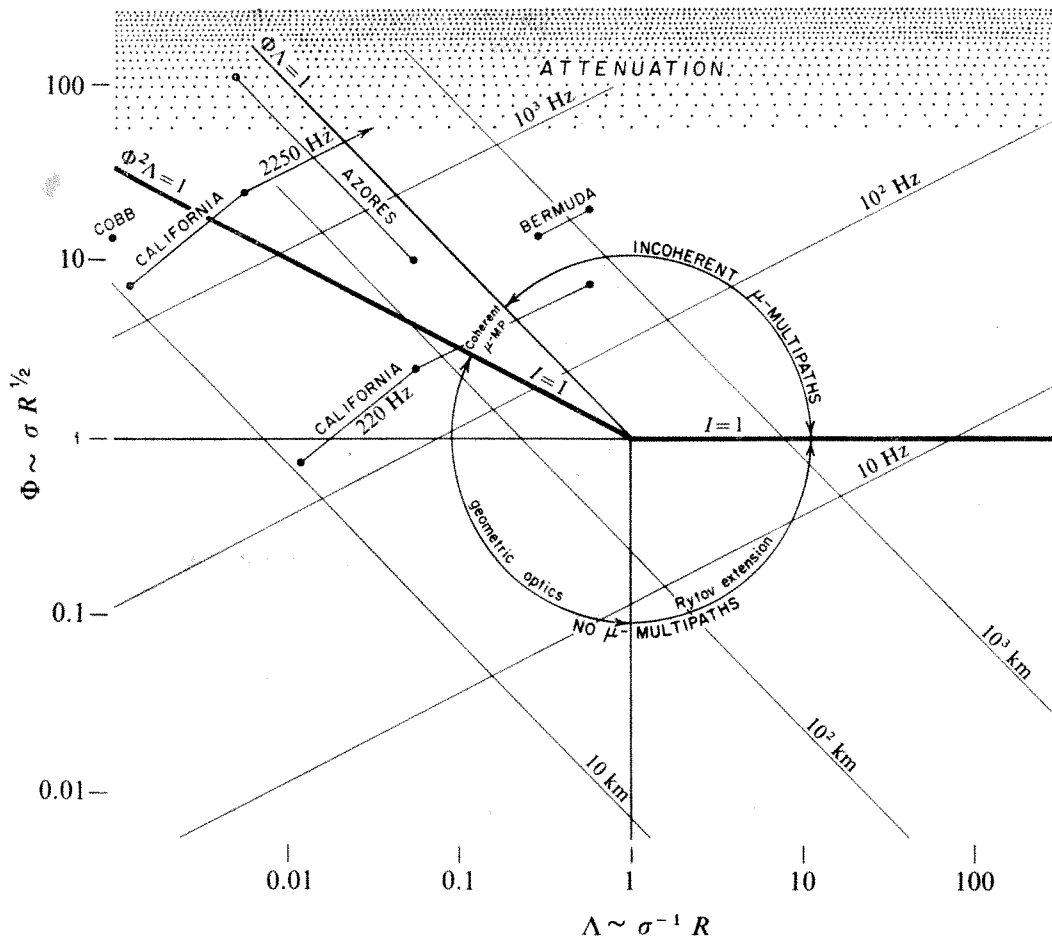


Fig. 3 According to the Dashen systematics, the character of acoustic transmission depends on the region in the Λ, Φ -diagram; Λ depends on the scale and Φ on the strength of the fluctuations in sound velocity. The Λ, Φ -parameters associated with various transmission experiments are shown. The functional dependence of the dimensionless coordinates on range R and frequency σ are indicated. For orientation, numerical values of R and σ are given for the special case of an upper path, and for scatter by a model ocean and model internal wave spectrum. (Accordingly, the actual Azores range of 35 km is inconsistent with the R -contours.)

What is measured is the vector sum of all paths, giving the intensity $I(t)$ and phase $\phi(t)$ of the combined multipath signal. The multipath $\langle \phi^2 \rangle$ is not the same thing as the singlepath $\langle \phi_1^2 \rangle$; it is generally larger and shows occasional near-180° 'jumps' associated with intensity fadeouts. Over a period of a month ϕ can change by many cycles, and it is necessary to keep track of the sign of the phase jumps. $\langle \phi^2 \rangle$ increases linearly with record time in accordance with random-walk statistics. (If one can measure travel time—for instance by measuring the group delay between two frequencies, or with broad band pulses—then the phase locus through the fade loses its stochastic property (personal communication from A. Ellinthorpe and T. Birdsall).) The spectrum of $\phi(t)$ contains high frequencies (associated with phase jumps) and low frequencies (associated with random walk) that are not contained in $\phi_1(t)$.

Even though the singlepath $\phi_1(t)$ is not directly measured, it can be inferred from the multipath statistics under quite reasonable assumptions³. The result is

$$\text{r.m.s. } \dot{\phi}_1 = 2.8 \times 10^{-3} \text{ s}^{-1}, 4.0 \times 10^{-3} \text{ s}^{-1} \quad (1)$$

for Mid-Station and Bermuda, respectively.

This parameter depends on the fluctuations of sound velocity along the transmission path, and can be calculated if certain statistical properties of these fluctuations are known. A statistical model of internal waves has been produced by Garrett and Munk⁴ from various oceanographic measurements (see ref. 5). An unexpected result was a high degree of universality with regard to time and place of observations, except perhaps in the close proximity of seamounts and other potential sources⁶. Internal wave motion dominates in the frequency band from cycles per hour to cycles per day. Munk and Zachariasen⁷ have computed the fluctuations in sound transmission for this particular model of ocean finestructure, and obtained

$$\langle \dot{\phi}_1^2 \rangle = 8\pi^{-2} \langle (\delta C/C)_{\text{axis}}^2 \rangle q^2 B R \omega_{in} n_0 \ln(n_{\text{axis}}/\omega_{in}) \langle j^{-1} \rangle \quad (2)$$

for the mean-square acoustic phase variations along an axial ray. Here n_0 and n_{axis} are values of the buoyancy frequency at the surface and sound axis, $\delta C/C$ is the sound velocity perturbation due to internal waves, q is acoustic wavenumber, B the scale depth of stratification ($dn/dz = -z/B$), R is range, ω_{in} is inertial frequency, and j is the internal wave mode number. An internal-wave-weighted average of j^{-1} equals $\langle j^{-1} \rangle = 0.44$. Equation (1) leads to the result

$$\text{r.m.s. } \dot{\phi}_1 = 3.5 \times 10^{-3} \text{ s}^{-1}, 5.2 \times 10^{-3} \text{ s}^{-1} \quad (3)$$

for the two stations. There are no loose parameters here, and the datasets underlying (1) and (3) are entirely independent, one acoustic, the other oceanographic. We would conclude that a connection has been made between the acoustician's ocean and the oceanographer's ocean. (A more detailed analysis allowing for non-axial ray paths leads to a somewhat lesser agreement.)

An interesting remaining question is whether the low-frequency $\phi(t)$ variations can be entirely ascribed to random-walk statistics, or whether they are in part the result of low-frequency ocean variations, such as mesoscale eddies.

Transmission over Cobb Seamount

Ewart⁸ transmitted 4 and 8 kHz pulses for about a week between source and receiver placed on Cobb Seamount at 1 km depth and 17 km separation. Ray-tracing gives a single downward refracted path with a turning point at 1,350 m. With this single-path geometry one can attempt to interpret the entire measured spectra of phase and intensity with those derived from an ocean model. Accordingly, much more can be learned than from the single parameter $\langle \phi^2 \rangle$ in a multipath experiment, at the expense, of course, of monitoring a much smaller ocean volume.

From the internal wave model one computes a phase spectrum bounded by the inertial and buoyancy frequencies, and proportional to ω^{-3} at intermediate frequencies^{7,9}. The measured phase spectrum has some of these characteristics. The measured r.m.s.

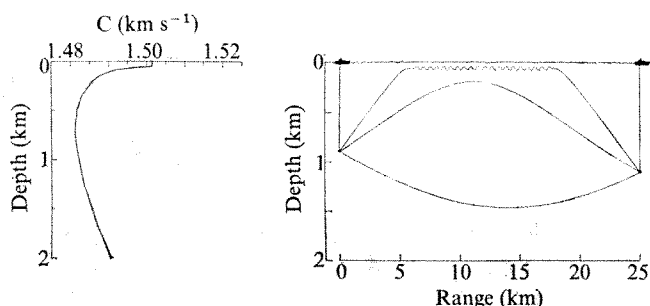


Fig. 4 Sound speed as a function of depth and associated refracted ray paths. A surface duct path is also shown (from ref. 16).

(ϕ) is 1.6 cycles, the value computed from the ocean model is 0.8 cycles. Again there are no loose parameters. But, the measured intensity fluctuations greatly exceed the computed fluctuations, particularly at high frequencies. The discrepancy has not been explained. It could be the result of ocean finestructure over the seamount which differs from typical open ocean conditions, although environmental measurements did not indicate a marked difference.

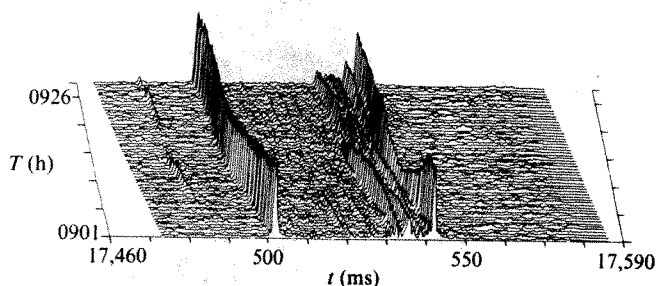
Acoustic experiment in the Azores

Ellinthorpe's¹⁰⁻¹² ambitious transmission study AFAR in the Azores involved a source at 550 m depth and a receiver at 750 m depth separated by 35 km. The refracted ray is limited by the source depth above, and the sound velocity maximum associated with the Mediterranean outflow at 800 m below. The experiment was conducted over a broad range of acoustic frequencies, and involved an intense program of ocean monitoring.

Interpretation of Ellinthorpe's experiment has gone well beyond the analysis of variance. For this purpose we need to refer to systematics developed by Dashen¹³. According to this systematics the signal statistics fall into five distinct classes, depending on the values of two parameters (Fig. 3). The diffraction parameter $\Lambda = (2\pi)^{-1} R_F^2 / L_V^2$ depends on the Fresnel radius R_F and the vertical coherence scale L_V , of the inhomogeneities. The strength parameter $\Phi^2 = \langle [q \int (\delta C/C) ds]^2 \rangle$ depends on the perturbations in sound velocity integrated along the ray path, with q designating acoustic wavenumber. In the lower region of Fig. 3, simple geometric optics and the Rytov extension apply. Ray paths undulate, but they are not split into micro-multipaths. Accordingly, the transmission time of an acoustic pulse will wander, but the pulse shape remains essentially undistorted. In the upper region the deterministic ray path is split into incoherent micro-multipaths, a transmitted pulse is severely distorted and spread, and C.W. intensity fluctuations are saturated in consequence of interference between these paths.

The Azores experiment is in an intermediate region where the intensity is only partially saturated. This, as with other inferences,

Fig. 5 Arrival amplitudes at 30-s intervals after high resolution processing (from ref. 16). Time is relative to the start of the transmitted pulse. Note the internal wave-caused relative complexity of the later, upper path arrival.



is in rather remarkable agreement with observations¹³, and give support to the hypothesis that the fluctuations are the result of internal waves. But most of the first-order statistics do not distinguish sensitively between an internal wave model and the Taylor hypothesis that the inhomogeneities are convected. But, some second-order statistics, such as the intensity covariance, are sensitive to this assumption, and the results are inconsistent with the Taylor hypothesis and consistent with an internal wave model¹³.

An experiment off California

We are preparing for a joint experiment¹⁴ to explore further the transition from unsaturated to saturated forward scattering. We will use multiple moored sources and receivers 250 nm offshore from southern California under a simple ocean geometry, that is, deep water and no seamounts. The moorings will be submerged and self-contained; the penalties for this relatively simple arrangement are the need for Rubidium clocks at each mooring and the need to monitor the mooring motions.

In terms of Dashen's systematics (Fig. 3), the planned diversity of frequencies (220 and 2,250 Hz), ranges (25–125 km), and paths (upper and lower) will cover the transition from geometric optics to coherent micro-multipath (2,250 Hz, SIO) and the transition from geometric optics to incoherent micro-multipath (220 Hz, WHOI). Associated ship-based listening stations will extend the 2,250 Hz observations to the incoherent micro-multipath regime. The experiment should provide a crucial test as to whether internal waves impose the fundamental coherence limits (space and time) to marine acoustics.

A second (and related) goal of this work is to assess the role of acoustic oceanography in synoptic monitoring of the oceans on a mesoscale (~ 100 km). It has become increasingly apparent in the last several years that most of the kinetic energy of the oceans is contained in mesoscale processes¹⁵. Mesoscale 'eddies' are analogous to atmospheric storm systems ($\sim 1,000$ km scale). This disparity in spacial scales makes the preparation of an 'ocean weather map' more difficult than preparation of the corresponding atmospheric map. A reverse disparity in time scales of ocean and atmospheric weather (~ 60 d compared with ~ 3 d) does not offset this difficulty. Acoustic oceanographic methods offer one possible mean for gathering data needed to map ocean weather.

The 2,250-Hz work off California is a preliminary step towards acoustic ocean monitoring. We will take advantage of the fact that the mean sound velocity profile gives one direct upper ray path and one direct lower ray path between two points located at about 1,000 m depth 25 km apart (Fig. 4). Travel time for reflected paths are well separated in time from those for direct paths and can easily be discriminated against. Very encouraging results from ship-based transmissions using this geometry (Fig. 5) have been obtained by Worcester¹⁶.

Changes in the oceanographic variables—temperature, salinity, and current—contribute to changes in travel time. Simultaneous transmission of pulses in opposite directions differentiates between current effects and those of temperature and salinity. Worcester has attained an absolute travel time precision of 10^{-4} s and a relative precision of 2×10^{-6} s, using modified matched filtering techniques. A fluctuation in travel time by 10^{-4} s corresponds to a temperature fluctuation of 10^{-2} °C, a salinity fluctuation of 10^{-2} ‰ or a current of 1 cm s⁻¹ integrated along the ray path. The 25 km averaging accomplished by the adopted mooring spacing is appropriate for detecting structures of eddy dimensions. We would, therefore, expect a distinctive travel time signature from the drift of a mesoscale eddy past the moorings. Identification of the contribution of individual variables to the overall travel time effect is as yet unsolved. A complete solution of this inverse problem probably requires data from many ray paths, each giving the result of its own vertically and horizontally weighted mean.

An environmental measurement program will accompany the acoustic work to compare results of the acoustic measurements

with data from traditional oceanographic instruments. A measure of the degree to which acoustic monitoring fills an unoccupied niche is the difficulty one faces in using conventional instruments to 'check' acoustically derived data. Even for our small, two-mooring experiment, we have not found it practical to monitor both acoustic paths with moored instruments. We will instead use conventional current and temperature meters to make continuous measurements along the simpler lower ray path only. Frequent upper path measurements will be made with instruments lowered from ships. Comparison of acoustic and 'spot' measurements will be limited largely to subinertial and tidal frequencies.

Acoustic monitoring of ocean weather has an advantage over conventional methods: by measuring between stations rather than at stations the information collected goes up with station number like $n(n-1)$ rather than n (allowing for reciprocals). There are no major technological problems. Indeed, for these subinertial frequencies one would dispense with the bothersome mooring position-keeping. Surface floats could allow for radio

control of clocks. The automated analysis of acoustic signals is a formidable task, but existing microprocessor technology is adequate. Experiments over the next few years will go far towards determining the feasibility of 'listening' to undersea weather.

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The life of the open sea

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"Third Fisherman—Master, I marvel how the fishes live in the sea.

First Fisherman—Why, as men do a-land; the great ones eat the little ones."

Pericles

THE living creatures of the near-surface waters of the open sea survive in one of the highly demanding and unforgiving habitats of the planet. Yet here a great and varied assemblage of creatures has evolved in or adapted into a vast realm that offers neither refuge nor obvious environmental niches to its myriad of inhabitants. The student of this region is strongly challenged by the very subtlety and obscurity of the adaptations and modes of existence, and often is astonished by his findings.

I will briefly review here some major aspects in the present understanding of the open sea, the nature of the forces and conditions that have set the range and compass of its progeny and will attempt to point out the curious and poorly understood steps that allow a relatively large population of highly developed, highly active animals to ensue from a food web initiated by microscopic plant cells, thinly dispersed in a dense fluid medium. I will speculate on defects in our present approaches towards understanding marine trophic (food web) systems and on an example of overlooked possibilities. Within the discussion, I will touch only briefly on adjacent interrelationships, coastal waters, shallow seas, coral shoals, and the environments of the deeper bathypelagic and benthic life. This short shrift to the boundaries of the environment that I discuss should be taken as a measure of the limitations of space rather than of importance, for the children of Tethys and the flocks of Nereus are, of course, no more isolated from the conditions and inhabitants of other realms than the disciplines of modern marine science can be cleaved one from another.

A properly structured discussion of any living system will begin with the processes by which the inorganic chemical and energetic sources enter and recycle in the system; and proceed with the living autotrophic entities that catalyse the elaboration of initial organic material from these lifeless sources. It will then describe the myriad and often

bizarre living forms and trace the Byzantine paths by which this organic material is utilised for its substance, processed for its energy, and finally returned again to the inorganic reservoir. I point out some developments that modify the classical visualisation of these steps.

Modern research largely affirms the broad aspects of the conventional concepts of the initial input of inorganic nutrients into the near-surface waters of the ocean. In this picture the special chemical requirements of phytoplankton as well as those common to almost all plant life are abundantly supplied only in those parts of the sea where some dynamic process provides the mechanical work required to recycle nutrient-rich water from deeper levels where they have been released, into the lighted upper layers where they are used. But, these sunny well-ploughed pastures of the sea, where luminous and chemical needs coincide, account for only a few per cent of the ocean's primary productivity, for they are of limited area. They exist mainly along eastern boundary currents, in the Antarctic, and at the equator, where the processes of upwelling hold sway. Because of the vast extent it is the great oceanic areas that account for most of the total productivity some 80%, despite the low production rates in these leaner waters¹.

It is clear, however, that the classical picture of the primary trophic inorganic steps needs to be modified in detail. Certain algal colonies (blue-greens) elaborate significant quantities of the essential nutrient, inorganic chemically-bound nitrogen². The recycling of nutrients within shallow seas and shelves, in coral atolls and other environments apparently contributes more to their productivity than previously supposed. In such environments, there may be only very low levels of plant nutrients dissolved in the water, for here recycling and uptake proceeds apace³, as in jungle soil. Recycling may also be internal, within animal tissues that bear captive plant symbionts, as in the case of corals and the *Tridacna* (giant) clams⁴. Hence classical chemical measurements of dissolved nutrient levels and inferences of productivity may be wholly misleading. Another process that modifies the classical portrayal seems to take place in the very clear waters of central oceanic gyres. Here in the uppermost

levels of the thermocline is a layer dense in chlorophyll, although the light level of less than 1% of surface illumination is below the minimum previously assumed necessary for net phytoplankton growth⁵. Apparently excess chlorophyll along with ancillary pigments or other adaptations allow phytoplankters to grow at the poorly illuminated diffusing upper edge of the nutrient-rich submerged waters—a process probably only general in the relatively steady conditions and clear near-surface waters of central oceanic regions.

Thus in the light of modern research, the general elements of the classical portrayal of the first step of the trophic system, primary productivity, survive quite well. Subsequent aspects of the conventional food-web picture seem to require more modification, however.

For example, dissolved organic material (DOM, the 'gelbstoff', refractory highly oxydised organic substances of high molecular weight) may assume an increasingly important trophic role. This material is undoubtedly the greatest of the organic reservoirs within the ocean, 200 times the total phytoplankton and particulate organic material⁶. Furthermore, its average age (3,400 yr) (ref. 7) argues that it is only utilised or removed slowly and perhaps only in some special conditions. Slow recycling of this great reservoir may be conducted by certain recently isolated bacteria of deep water which can utilise it⁸. It may become rapidly available along the hydrothermal areas of deep rift zones at oceanic ridges, where heat, pressure, and brief reducing conditions may transform it into more available chemical forms. Wherever it is that DOM is recycled, food input could be very large. For example, the rate at which DOM becomes available in the North Pacific could exceed the primary productivity there by a factor of 10 (ref. 9).

Other modifications of the trophic picture emerge apace, and I will sketch only a few. Remarkable discoveries of the nature of metabolites and organic pathways include the demonstration of a bewildering range of halogenated organic products (a chemical province recently thought to be the chemists' exclusive domain) elaborated by marine plants for various defensive, offensive, and possibly communicative purposes¹⁰; the dominant role of waxes in major parts of the food webs of the open sea and coral reefs¹¹; the role of the ubiquitous behaviour of the active pelagic zooplankton and small nectonic (more strongly swimming) organisms in performing a diurnal round-trip migration from the surface to mid-depth, thus providing a number of functions—including leading them to productive areas¹²; the demands of energetics that force large active predators preferentially to select surprisingly small organisms for their major food resource¹³; the well-nigh invisible environmental niches provided for apparently fully equivalent species by their different responses—not to conditions—but to the time rate of change of conditions¹⁴; the remarkable chemotactic-motor trail-following behaviour of some marine organisms¹⁵; and many others.

In this last example, sergestid shrimp have been shown to follow a fine thread-like trail of amino acids with ultimate swimming speed and preciseness, an astonishing display of chemotactic motor control.

These examples epitomise the surprising discoveries that the observation of the forms, behaviour, and processes of life of the sea will ever yield up to its students. At the same time the same examples may also indirectly point to one of the deficiencies of the underlying observational, scientific, and philosophical approach to the trophic pathways of the open sea and perhaps to other aspects of marine biology as well.

The careful and thoughtful observations and measurements of living forms, behaviour and pathways have taught much important understanding in forest and grassland¹⁶ and in some estuarine, near-shore and other

environments. Yet in the open sea, and despite the inherent fascination of naturalists towards the activities of the creatures of the sea, many of the dominant activities and pathways are fundamentally not discernible. Major pathways of detritus, reproductive products, growth and dissolved organic material are virtually invisible, yet the delusion commonly persists that with only sufficient collections, observations, measurements and analysis, the full dynamic nature of the food webs of the open sea eventually will stand revealed. I will enlarge briefly on this, for it may well be the principal diastema in our philosophical approach to the trophic systems that dominate two-thirds of this planet.

The flow of food from initial microscopic levels of the open sea to higher forms such as fishes, squid, and marine mammals seems to be dominated by quite a different process from similar fluxes on land or near-shore and shallow aquatic environments, where the principal inputs are by large plants¹⁷. In the pelagic realm a sort of food web composed of the progeny of many species seems to constitute a major pathway of material to the larger forms. This pathway is dominated by growing larvae and juveniles, which suffer immense mortality in their role. It is a sort of huge polyspecific extension and exacerbation of the 'Faginism' observed in some cases. For example, in lakes that have been stocked with one species of predacious fish, a food web develops from successive predation of juveniles by larger juveniles ultimately supporting a few reproducing adults. Such a system is in sharp contrast with other food webs where large plants provide the initial input, large herbivores are supported, the populations are dominated by adults, and mortality is low.

Undoubtedly this system has developed in the open sea for access of the larvae and juveniles to the abundant microscopic and near microscopic food at the commencement of the food web. It is responsible for the immense flow of food material as reproductive products (egg, sperm, and larvae) from the higher forms down towards the beginnings of the system, and may be largely responsible for the very existence of higher forms in the open sea.

It is interesting to speculate on the role of those marine creatures that take from but do not contribute to these essential pathways. The terrestrial expatriates (marine mammals, birds and reptiles) and some sharks which bear large young, depend entirely on the peculiar pathways of the pelagic food web but escape from both the associated contribution to and the predation within the system upon which they are dependent. Any substantial dominance of these forms may thus lead to collapse of the entire higher pelagic system. This fundamental instability of the higher levels of the pelagic food web may illuminate events in the palaeontological record where explosive proliferation of highly-developed (and apparently viviparous) pelagic predators culminated in their own universal mass extinctions and that of many other higher pelagic creatures¹⁸.

The present marine mammals and tuna-porpoise policies need to be restudied in light of this possibly fundamental long term ecological instability of the pelagic system.

I will conclude with an added example of each of two principal themes on our understanding the conduct of living system of the open sea, one philosophical and one observational.

First, there are opportunities to view trophic systems by overleaping the details of processes with the same philosophy that enables us to coalesce a microcosm of molecular kinetic complexity into a single meaningful measure—temperature. Such an approach complements the more detailed naturalistic descriptions and measurements. One such model views organisms as operators that continually distribute and redistribute resources into a few specific reservoirs by appropriate transfer coefficients¹⁹. These reservoirs are, most simply, living material, dead

retrievable material, dead irretrievable matter, and reproductive products. The approach generates useful and meaningful expressions of potential relative biomass of trophic types and other trophic relationships, and seems to explain a number of anomalies, such as the frequent observation that the biomass of predacious forms exceeds that of the herbivorous forms with which they are in an apparent equilibrium. It also provides reasons for both the general immunity of marine organisms from the bioaccumulation of trace substances in the food web (including pollutants); and the vulnerability of marine birds and mammals to the bioaccumulative process.

As a final speculative example, aggregation of the active creatures of the open sea, such as schooling, is an almost universal feeding, defensive, predatory, or mating strategy. Indeed, the existence of both baleen whales and marine fisheries is almost wholly dependent on species that display strong schooling behaviour. Nowhere is schooling more conspicuous than in the small filter feeding fishes, the clupeoid sardines, anchovies, herrings and their relatives. It has long been known that schools of such fish are strong scatterers of underwater sound and easily detected by sonar devices. The strong sonar reflection of these fish is due to the abrupt discontinuity in sound velocity at the boundaries of their gas-filled swim bladders. Although underwater sound of the frequency and wave length in 'fish-finders' responds to the presence of a swim bladder as though it were a discontinuity in the water, lower frequency sound will respond to the swim bladder as a property of the medium. This is quite properly analogous to the scattering of light by fog and the penetration of such obscuration by radar.

Thus, 1,000-cycle (1 kHz) underwater sound with a wave length of almost 2 m will envelope a number of fish in the school per wave length. Simple calculations show that the velocity of low-frequency sound within a fish school can be as low as one-third the velocity in sea water. Such a change in sound velocity is orders of magnitude greater than that engendered by variations in the ordinary physical-chemical conditions of temperature, salinity, and

pressure. Thus to low frequency sound the fish school is a wholly unique object, and such sound must focus abruptly within the schools. A consequence of the schooling of small fish is thus the creation of an immensely powerful, complex and continuously pliant lens for the focusing of low frequency sound, a lens that is composed entirely of detectors of such energy! Here is an example of the development of certain qualities (for example, a hydrostatic device) and particular behaviour (for example, schooling) under quite disparate requirements interacting to form a wholly new advantageous property, which could not be approached at the level of mutation of the individual organism. In this case the serendipity is the creation of a unique living lens to magnify and respond to the sounds of distant predators!

In this brief resume of the life of the open sea, I have suggested some of the modifications of the classical picture emerging from the observations and the burgeoning new insights into its forms and processes; and some of the major deficiencies in the present approaches and philosophy.

I have also speculated on examples of the possible future advancements of understanding that the instructor, Nature, may ultimately teach us within the reaches of the great realm that encompasses two-thirds of the surface of this planet, the open sea.

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Diversity and faunal composition of the deep-sea benthos

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Improved techniques have demonstrated a remarkably high species diversity in the deep sea. Long-term environmental stability has resulted in establishment of diverse communities whose species have become biologically accommodated to each other. The deep-sea meiofauna (about 60–500 µm) mainly consists of foraminiferans and nematodes. The macrofauna is dominated by polychaetes, peracarid crustaceans, and bivalves. The major components of the megafauna are brittle stars bathyally, large scavenging fish and amphipods abyssally, and amphipods hadally. Biomass and numerical density decrease rapidly with distance from land. Biomass seems to be of the same order of magnitude for the three size groups, while meiofaunal density in the upper bathyal zone may be up to 1,400 individuals per 10 cm² and macrofaunal density 20,000 individuals per m².

In the last decade, modern techniques have led to many remarkable discoveries regarding life and life conditions in the deep sea. Rather than attempt a general brief survey of new knowledge gained in almost all fields of deep-sea biology, I have preferred to concentrate on a limited number of topics which represent some of the most striking advances in our conception of deep-sea life.

Species diversity

Until the 1960s it was the general experience that with increasing depth and distance from land the benthic fauna consisted of fewer individuals belonging to fewer species. The recent introduction in deep-sea research of the anchor dredge¹, the epibenthic sled², and careful washing of the samples through fine-meshed sieves drastically changed this concept. Greatly increased numbers of individuals now became available, numerically increasing the number of species, and also allowing the taxonomists to recognise that the samples contained numerous closely related species.

The high species diversity was first demonstrated in the

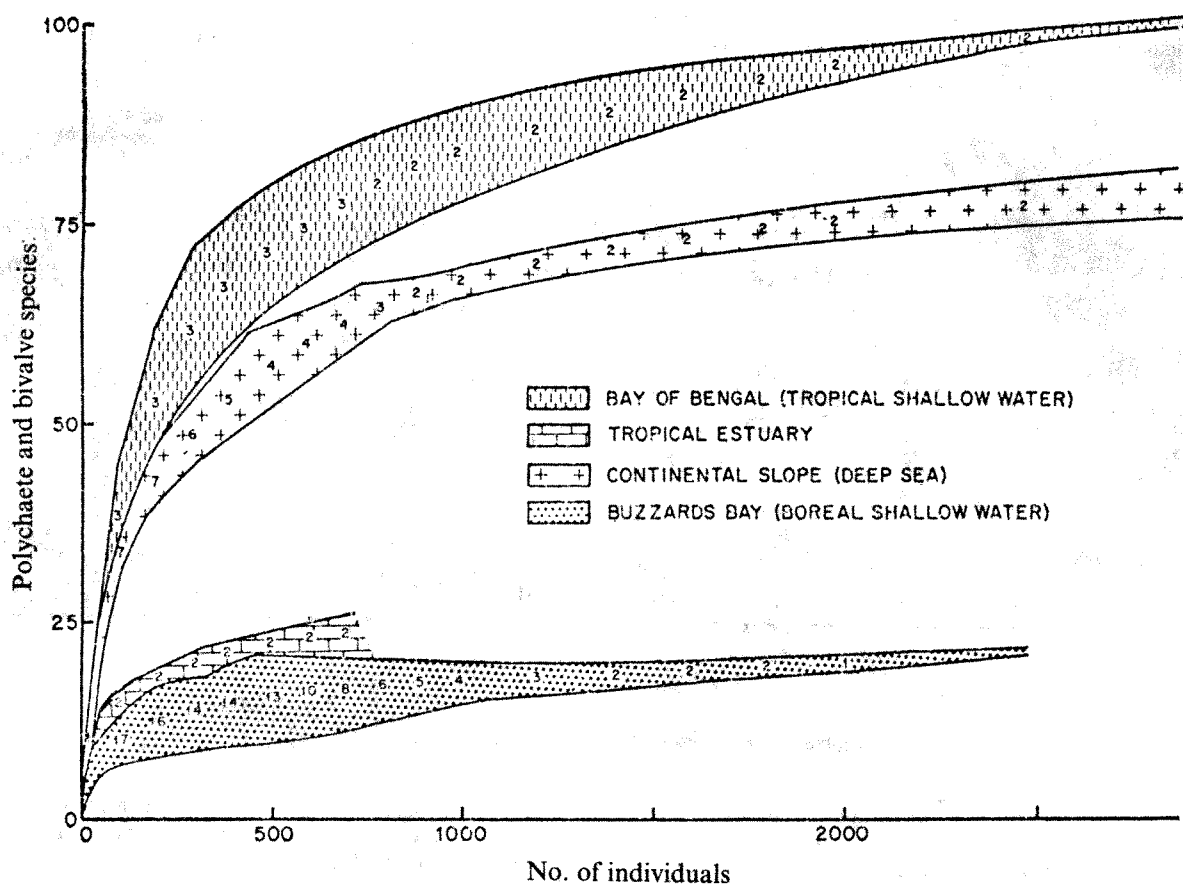


Fig. 1 Range for diversity values found for three shallow-water and two combined deep-sea environments (the latter in the NW and the SW Atlantic, 300–2,500 m). The figures in the bands indicate number of samples. (Slightly modified from ref. 6.)

north-western Atlantic². Samples subsequently taken off western Europe, in four basins off West Africa and in the Brazil Basin, collected and processed with similar methods, show the same general pattern.

For instance, three sled samples from the north-western Atlantic from 1,400, 2,900 and 4,700 m contained 25,200, 12,100 and 3,700 individuals belonging to 365, 310 and 196 species, respectively². The hitherto most successful deep-sea sample was taken with a large trawl in the East Pacific at 3,600 m and contained 2,100 individuals belonging to 132 species³. Among the dominant groups, the greatest diversity is invariably found in peracarid crustaceans, followed by polychaetes and bivalves, while ophiuroids are always represented by few species. If there is one species of brittle stars per 1,000 individuals, there are four species of bivalves, 10 of polychaetes, and 17 of peracarid crustaceans⁴. Also harpacticoid copepods, an important component of the deep-sea meiobenthos, show high diversity⁵.

In order to compare deep-sea diversity with that in shallow water, soft-bottom samples were obtained from, among other places, a boreal and a tropical estuary and a boreal and a tropical marine environment, and processed with the same techniques. Based on the polychaete–bivalve fraction of the fauna, which accounts for about 80% of the total fauna in most infaunal samples, and using a rarefaction methodology which allows direct comparison of samples with differing numbers of specimens, Sanders^{6,7} showed that only in the physically stable, shallow, tropical marine environment was there a diversity as high as that of the deep sea (Fig. 1). Conversely, a low diversity is characteristic in environments where physical parameters (for example temperature or salinity) fluctuate greatly.

Basic to Sanders' stability–time hypothesis is the idea that long-term environmental stability has allowed a highly refined competitive niche differentiation. Since food is the

most important limiting factor in the deep sea, each species may have evolved a high food specialisation, thereby avoiding competitive overlap with other species of the community.

Although they do not deny the presence of niche specialisation, Dayton and Hessler⁸ believe that continuous and intense biological disturbance by predators is the main element responsible for controlling population size and maintaining high diversity. They find that in the deep sea a high degree of food-niche specialisation is unlikely. There can be no sharp demarcation between deposit feeders and predators, because in order to survive, both categories must possess a certain flexibility in their food choice. An appropriate term for animals of such a broad feeding-type is 'croppers', that is animals of any size which consume live food, either alone or in combination with dead or inorganic materials. The pressure exerted by croppers on populations of smaller animals reduces the probability of competitive exclusion of the latter and allows a high overlap in the utilisation of food resources.

Grassle and Sanders⁹ think that in view of the known life history characteristics of deep-sea species, predation cannot be the principal mechanism for controlling population size. Recent investigations of numerous individuals belonging to single species of the tan-aid family Neotanaisidae, of the isopod family Desmosomatidae, and of protobranch bivalves showed small brood sizes and that each population is characterised by a much higher proportion of adults compared to young stages than encountered in comparable shallow-water species. Moreover, growth rate is slow and longevity high, as dramatically demonstrated by Turekian *et al.*¹⁰, who by using ²²⁸Ra chronology determined the age of a protobranch deep-sea bivalve, measuring up to 8.4 mm, to be about 100 yr. Small population size, low reproduction and mortality

Table 1 Composition of the macrofauna (% of the total number of individuals) at bathyal depths off Southern California and in the Arctic Ocean, at abyssal depths in the Atlantic and the Pacific, and at hadal depths in the Kurile-Kamchatka, Japan, Kermadec, and Java Trenches.

Gear	Core samplers		Anchor dredge			Epibenthic sled			Trawl
Source	Ref. 13	Ref. 18	Ref. 11			Ref. 2			Ref. 19
Area	Californian basins	Arctic Ocean	North-western Atlantic		Central N. Pacific	North-western Atlantic			Trenches
Depth (m)	1,130-1,230	1,060-2,530	300-3,750	4,400-5,000	5,600	1,400	2,500-2,900	4,700	6,200-10,000
Group									
Polychaeta	76	50	70	55	55	43	5	8	7
Crustacea Peracarida	14	9	7	33	24	17	20	32	5
<i>Tanaidacea</i>	4	3	2	19	18	4	1	1	<1
<i>Isopoda</i>	3	5	1	12	6	6	6	18	4
<i>Amphipoda</i>	5	...	4	2	...	4	3	5	<1
Bivalvia	2	9	13	4	7	18	5	47	19
Echinodermata	3	<1	<1	1	1	11	67	2	57
<i>Ophiuroidea</i>	2	<1	<1	<1	<1	5	64	2	2
<i>Holothurioidea</i>	<1	<1	<1	...	<1	5	3	...	54
Others	4	31*	9†	8†	12‡	2	3	11§	11
No. of individuals	5,164	167	28,277	681	287	25,242	31,405	3,737	21,589

*Mainly tetraxonid sponges.

†Mainly sipunculids.

‡Mainly oligochaetes, scaphopods, and bryozoans.

§Mainly gastropods.

||Mainly pogonophorans due to a mass occurrence in one sample from the Kurile-Kamchatka Trench.

rates, and proportionately modest numbers in the smaller size groups would neither be expected nor have high survival value in predator-controlled communities or in any environment where transient diversity is important. These characteristics would, however, be expedient where long-term or evolutionary diversity is a product of past biological interactions in a physically stable environment such as that of the deep sea.

In order to arrive at a better understanding of the causes of deep-sea diversity, other methods must be applied, involving, for example, detailed analyses of microdistribution, nutrition, biochemical specialisation, and predator-prey relationships.

Construction of the box core sampler covering an area of 0.25 m² and partitioned into 25 subcores, each 100 cm², has made it possible not only to obtain quantitative data comparable with those obtained by bottom grabs (see below), but also to evaluate the microdistribution.

A comparison of core samples from the oligotrophic sea floor at 5,600 m in the central North Pacific Gyre showed a diversity of polychaetes and crustaceans exceeding that of sled samples from the oligotrophic north-western Atlantic¹¹. This high diversity in an extremely oligotrophic environment indicates that sparseness of food does not affect diversity.

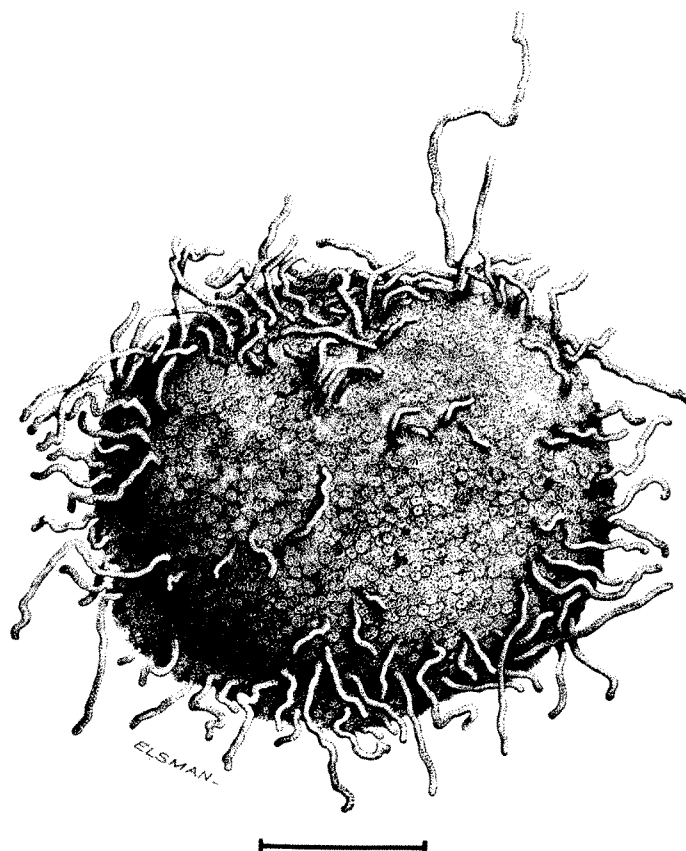
Jumars¹² studied the polychaete fauna in partitioned box core samples from 1,230 m off Southern California and found that a uniform dispersal dominated within cores. Thus, the extremely high diversity observed cannot primarily be due to patchiness or spatial heterogeneity. But, species assumed to be sedentary (small-ambit species) were more diverse than species assumed to be mobile and thus having larger ambits. This indicates that microhabitat specialisation, acting on spatial scales smaller than 100 cm² (the area of the subcores), here has a major role in maintaining high polychaete species diversity. Similar results were obtained by comparing the entire fauna in box cores from two bathyal basins off Southern California¹³.

Composition and biomass of the deep-sea benthos

The grouping of benthic organisms into size classes reflects the sampling and processing techniques. Thus, it is generally accepted that the meiofauna comprises animals which are

retained on screens of about 60 µm and pass through screens coarser than 300-500 µm. The macrofauna is retained on the latter screens, while the megafauna consists of larger animals which cannot be collected adequately with a grab or core sampler for quantitative evaluation.

Fig. 2 One of many komoki from 6,100 m in the central North Pacific. It belongs to a new superfamily of agglutinating foraminiferans which have been found to be extremely abundant in the deep sea. Scale bar 1 mm. (After ref. 21.)



Most of these animals are large enough to appear in survey photographs.

The meiofauna: The number of living foraminiferans varies considerably regionally and with depth. Highest numbers (up to 1,400 per 10 cm² and 1 cm sediment depth) occur at bathyal depths off Southern California, while the mean numbers in the East Atlantic are an order of magnitude lower¹⁴. This is also the case in the Kurile-Kamchatka area, with highest numbers at bathyal and hadal depths and lowest numbers abyssally¹⁵. In contrast to other meiofaunal components (except to some extent the nematodes), a significant proportion of foraminiferans (up to 60%) lives in sediments deeper than the uppermost 1 cm (ref. 14). Calcareous foraminiferans dominate above about 2,000 m, while agglutinating forms are the sole components deeper than 3,500–4,500 m.

Comparisons of number of foraminiferans with the total meiofauna range from 2–27% foraminiferans off Mauritania at 250–4,500 m (ref. 14) to 2–90% (mean 61%) at 250–2,500 m (ref. 16) and 65% at 4,000 m (ref. 17) off North Carolina. In biomass, foraminiferans constitute 15–86% (mean 53%) in the Arctic Ocean¹⁸.

Except for local abundance of foraminiferans, the overall dominating group is the nematodes, in numbers as well as in biomass. Excluding foraminiferans, nematodes constitute 39–96% (mean 85%) of the meiofauna in the 165 compared samples and subsamples so far investigated from 11 areas at bathyal and abyssal depths. Dominance of nematodes does not seem to vary with increasing depth or distance from land.

Second in importance numerically are harpacticoid copepods, followed by polychaetes, ostracods, and crustacean nauplii. Rotifers, oligochaetes, bivalves and tardigrades may be present.

The density of organisms varies greatly, from 0.3 individuals per 10 cm² in the abyssal oligotrophic central North Pacific to 1,000–1,400 individuals per 10 cm² on the East and West Atlantic continental slope. The lowest biomass recorded is 0.1–0.05 g m⁻² surface and 4 cm sediment depth in the abyssal Mediterranean and the abyssal central North

Pacific, the highest is 3.5–6 g m⁻² at bathyal depths in the North Atlantic. At abyssal depths the biomass may be as high as 2.8 g m⁻² (the Norwegian Sea) and is usually 0.2–0.3 g m⁻².

Both numerically and in biomass there is a significant difference between the macro- and meiofauna with increasing depth. The marked macrofaunal decrease is not typical for the meiofauna. As mentioned above, meiofaunal biomass may be remarkably high at abyssal depths. Transect investigations in seven areas of the Atlantic¹⁴ show a maximum decrease of meiofauna biomass of 16–83% mean (50%) between upper slope and 3,000–5,000 m.

The macrofauna: A detailed analysis of 10 box core samples from two basins off Southern California (1,200 m) showed that polychaetes constitute more than 75% of the total number of individuals (Table 1). Next in importance are the peracarid crustaceans, with an almost equal share between amphipods, tanaids and isopods. Rather insignificant are bivalves and echinoderms (mainly consisting of brittle stars). In 75 quantitative samples from mainly bathyal depths in the Arctic Ocean half of all individuals (minus nematodes) were polychaetes (Table 1), followed by tetraxonid sponges (13%), crustaceans, and bivalves.

Also in abyssal communities, polychaetes, crustaceans, and bivalves generally are the main components of quantitative samples. In Table 1 the relative number of individuals in the marginal and oceanic north-western Atlantic and in the central North Pacific Gyre are compared. Polychaetes dominate, although in the oceanic environments they decrease somewhat in importance. The same applies to bivalves, while crustaceans, particularly tanaids, become proportionately more abundant. Combined, the three groups constitute 86–92% of the total fauna.

The above comparisons are based on box core and anchor dredge samples of the infauna. In epibenthic sled samples from the north-western Atlantic, which consist of both epifauna and infauna, a rather similar relative abundance prevails bathyally (Table 1), but at abyssal depths the composition is different. Polychaetes have an

Fig. 3 Quantitative distribution of zoobenthos (wet weight biomass) in the world oceans. (Slightly modified from ref. 23)

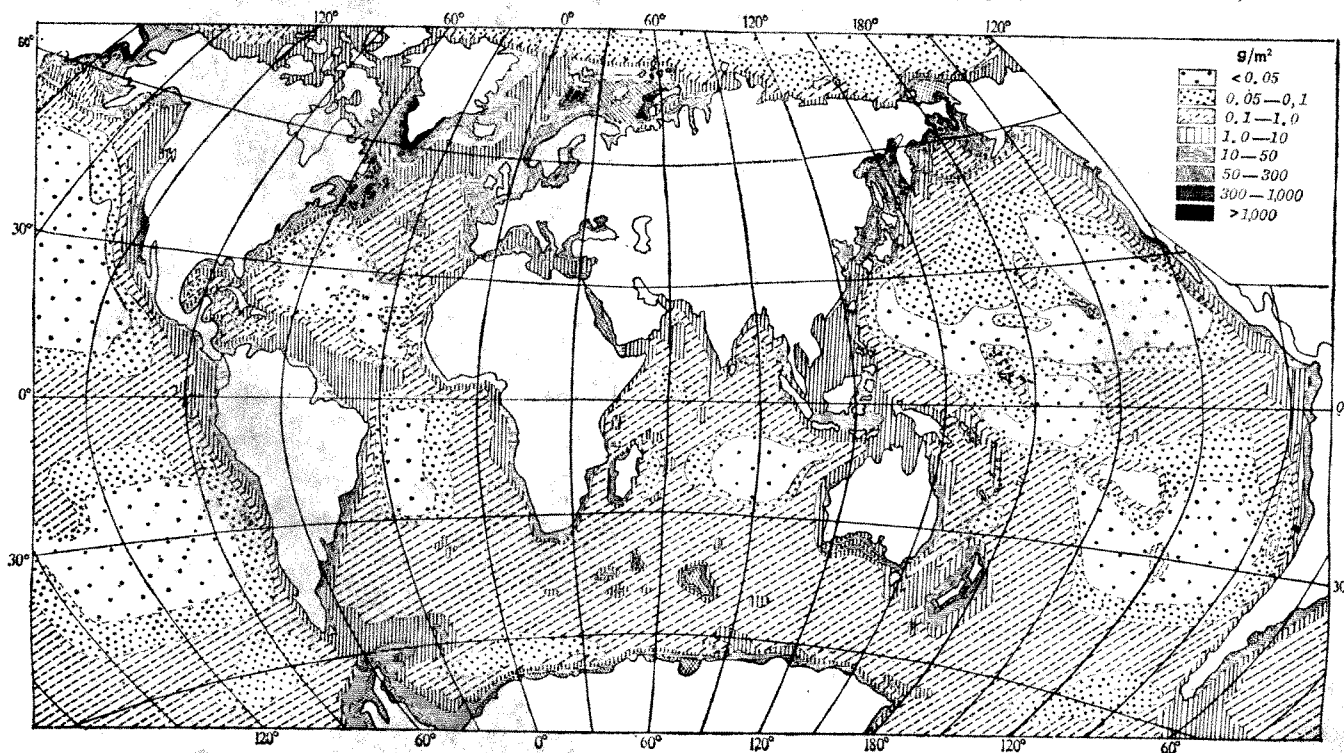




Fig. 4 About 3 m² of the sea floor at 1,800m depth SE of Massachusetts, USA. Alvin dive 685, 1976. Many brittle stars, *Ophiomusium lymani* (some in motion), white sea urchins, *Echinus affinis*, and one holothurian, Synallactidae. (Courtesy of Linda Morse, WHOI.)

inferior role. Brittle stars are very abundant in the more productive environments closer to the shore and are followed by cumaceans (another group of peracarid crustaceans). Bivalves dominate in the deep oligotrophic environment under the Sargasso Sea, followed by isopods. In Arctic Ocean sled samples from bathyal depths, sponges and polychaetes constitute nearly 25% each and crustaceans and ascidians 15% each¹⁸.

An evaluation of the composition of the hadal trench fauna cannot yet be based on quantitative data. Instead, Table 1 shows the average relative abundance of individuals collected in sled trawls in the four most thoroughly investigated trenches (which have an almost equal relative abundance). In these, and in all other trenches explored so far, the dominance of holothurians is as high as that of the polychaetes bathyally and in core/grab samples. Holothurians are followed in abundance by bivalves, polychaetes, and isopods. The preponderance of bivalves here and at 4,700 m in the north-western Atlantic (Table 1) may be due to winnowing during sampling at such great depths, resulting in loss of some of the lighter elements of the samples.

Two groups of protozoans of macrofaunal size, both agglutinating rhizopodes, are remarkably abundant, at least in some areas. The xenophyophores which are usually several centimetres in diameter may have pseudopodia extending 10–15 cm (ref. 20) and thereby be of great ecological importance. The other group is a hitherto overlooked, new superfamily, the Komokiacea (Fig. 2). These have a world-wide distribution, with a high relative abundance in hadal trenches and in the abyssal oligotrophic North Pacific. In the latter area their volume exceeds that of the metazoans and equals that of other foraminiferans²¹.

Extensive quantitative investigations in the Pacific²² show that polychaetes also constitute the dominant group at abyssal depths in biomass (up to 50%), although with the same decrease in importance in oligotrophic areas as was demonstrated when considering numerical abundance. Furthermore the biomass of crustaceans is relatively high in the oligotrophic areas (up to 25%) but almost obsolete in the productive areas. Similarly, echinoderms vary greatly in relative abundance and are nearly as dominant in weight in the south-western Pacific as the polychaetes. In the Arctic Ocean¹⁸, bivalves dominate in biomass (58%), and sponges (16%) are also more important than polychaetes.

The data of quantitative investigations demonstrate a pronounced decrease in total biomass with increasing depth

and distance from land, reflecting the reduced food input (Fig. 3.) Thus, 50 miles off California at about 200 m depth the total biomass is about 40 g m⁻², 50 miles further out, at 4,400 m, the biomass drops to 3.5 g m⁻²; a further decrease to 0.2–0.5 g m⁻² occurs 600 miles from the coast at about the same depth²².

In samples consisting of many small, equal-sized animals, numerical density is a better measure for biological biomass than the ordinary wet weight measurements which include inorganic shells of bivalves and skeletons of echinoderms and crustaceans. Comparable numerical densities are available from the north-western Atlantic and show the same marked decline, from 21,000 individuals per m² at the edge of the continental shelf and between 8,600 and 1,700 on the slope to only 30–150 individuals at 5,000 m under the Sargasso Sea and 200–700 on the slope of the oceanic Bermuda island group¹.

Exceptions to the general rule of macrofaunal decrease with depth and distance from land are the Arctic and Antarctic Oceans and deeper water off upwelling areas with a particularly high production in the euphotic zone. A relatively rich fauna also exists in most trenches close to continents and larger islands, probably because the trenches act as traps for organic material. Biomass as high as 40 g m⁻² has been reported from the Aleutian Trench²².

The megafauna: Photographic documentation, combined with trawling or collecting from submersibles, are recently introduced methods which supplement conventional trawling, thus allowing a more detailed and reliable evaluation of the composition and distribution of the epibenthic megafauna.

Series of bottom photographs from bathyal depths in the north-western Atlantic off the Carolinas²⁴ show dominance

Fig. 5 Grenadiers (macrourids) and possibly brotulids attacking bait on an extremely oligotrophic bottom, covered with manganese nodules, at a depth of 5,900 m in the central North Pacific. The width of the picture equals about 2 m. (Courtesy of John D. Isaacs, SIO.)



of the quill worm *Hyalinoecia* (up to 15 individuals per m² at 450 m) and the brittle star *Ophiomusium* (2–6 per m² at 1,700–2,200 m). Although nearly half of the identifiable species are echinoderms, only one other brittle star is abundant. Both brittle stars have a wide vertical distribution, while most other megafaunal species show the same narrow zonation as macrofauna species⁴.

Ophiomusium and *Hyalinoecia* also dominate at similar depths off New England²⁵ (Fig. 4). Except for a sea urchin, the other 28 identifiable species (17 of which are fish) are rare. At about 2,000 m the megafaunal numerical density is three orders of magnitude less than the macrofaunal density in anchor dredge samples.

Calculation of the biomass of the megafauna components in trawl samples from the same area²⁶ showed that macrofaunal and megafaunal biomass are, however, of the same order of magnitude. The most abundant taxa are fish and echinoderms. Generally fish dominate in number and particularly in weight at all depths (140–1,900 m), and their size increases with increasing depth.

In recent years an extraordinary abundance of large, mobile fish and other vigorous scavengers has unexpectedly been demonstrated in the deep sea by means of baited ballast and a camera suspended above the bait. Every 10 min a still photograph or motion pictures are taken, and after 24–48 hours the camera is automatically released and returned (using floats) to the surface.

This free-vehicle research has been conducted at almost all depths in all main oceans^{8,27}. At bathyal depths the usual sequence of events is first the appearance of large shrimps, brittle stars, and amphipods, followed about 30 min later by a variety of fish which often suddenly scatter because a very large sleeper shark (*Somniosus*) moves in. Crabs, sea urchins, snails, and so on are responsible for the final cleaning.

At abyssal depths fish and amphipods are the overall dominating scavengers. Large grenadier fish (*Coryphaenoides* and other macrourids) are very active (Fig. 5), while eelpout-like fish (Lycodidae) and brotulids remain almost motionless for a very long time or at most nibble gently at the bait. Lysianassid amphipods up to 28 cm long (twice as long as any amphipod previously known) were photographed at 5,300 m in the North Pacific²⁸ and at 7,000 m in the Peru–Chile Trench (Fig. 6). At 9,600 m in

Fig. 6 Multitude of amphipods and several shrimplike mysids gather around the bait at 7,000 m depth in the Peru–Chile Trench, stripping the bait in a few hours. (Courtesy of John D. Isaacs, SIO.)

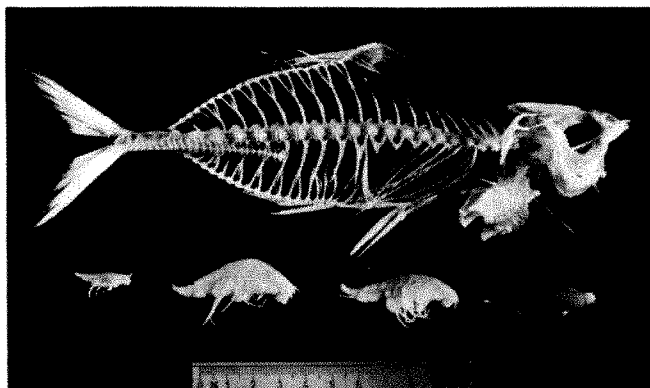


Fig. 7 A thoroughly skeletonised bait fish and four of thousands of large, ravenous amphipods from traps set at 9,600 m depth in the Philippine Trench.

the Philippine Trench thousands of amphipods were attracted to fish bait, and subsequently more than 10 litres were caught in baited traps (my experience in 1975 on board R/V Washington of Scripps Institution of Oceanography). All belonged to one species of Lysianassidae (Fig. 7), of which only four specimens had previously been collected in the trench²⁹.

It is remarkable that scavenging fish and amphipods are particularly abundant in the central North Pacific Gyre, where the benthic standing crop is extremely low^{11,22,23}. The ubiquitous scavengers must therefore rely on large 'food parcels' descending from above, for example, dead bodies of fish and marine mammals and food fragments from surface-feeding fish. Once on the bottom, the food is quickly located and consumed by opportunistic scavenging fish and amphipods.

Amphipods caught in baited traps in the North Pacific³⁰ and in the Philippine Trench were all without broodpouch plates or eggs in spite of their large size. This fact suggests that onset of reproductive maturity may be conditioned by the intake of a large meal³⁰ and may be postponed until one such is available.

Nothing is known about the density of scavengers or the distance from which they are attracted to food. Obviously, in order to sustain this roaming fauna, larger amounts of 'food parcels' must reach the bottom than previously anticipated. In view of the abundance of scavengers, the faeces they disperse over the sea floor is probably an important source of energy for deposit feeders⁸, thus contributing to the maintenance of life in the deep sea.

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Drugs from the sea—fact or fantasy?

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Drugs from the sea are as much a potential marine resource as cultivated fish, and mineral deposits. The study of the chemical structure and properties of unusual metabolic products of marine life is a subject where marine ecology and the experimental sciences of chemistry, biochemistry, pharmacology and medicine share a common and complementary interest. The development of ad hoc collaboration between specialists has advanced basic knowledge and resulted in a significant feedback to marine biology and ecology as well as in the development of some useful drugs.

OUR present armamentarium of drugs used to treat man and his domestic animals is mainly comprised of either natural products of unusual chemical structure and function derived from terrestrial microorganisms, plants and animals, or synthetic analogues of these products. Evolutionary adaptation has presumably resulted in metabolic diversity and cellular specialisation for their biosynthesis. In some cases it has been shown that these bioactive products of metabolism represent one mechanism of species survival in the highly competitive situations that can arise in natural ecosystems¹.

The clinical success of sulphonamides (Prontosil) and penicillin in the period 1938–45, led to a general acceptance of the idea that many human diseases would be amenable to treatment with selectively toxic chemicals. This led to the subsequent and successful search for new drugs. There is now some concern about the diminishing returns resulting from a continued emphasis on the screening of terrestrial life forms and a developing interest in the certainty that substances of therapeutic importance are to be found in the vast and diverse range of marine life—an estimated 500,000 species in 30 phyla. This certainty arises from the numerous reports of the pharmacological and toxicological effects elicited by extracts of marine organisms and from our knowledge of substances of ecological importance, such as the potent venoms and poisons concerned with offence and defence reactions. There is also a very extensive folklore and old records of a more or less anecdotal nature that are relevant to both human medicine and marine ecology^{2–4}. In this article, actual and potential therapeutic agents have been broadly classified as either 'antibiotics' that may be used to selectively control and destroy pathogens and parasites that cause disease, or systemic drugs that can modify or control body metabolism and function.

Antibiotics

Marine bacteria, fungi, phytoplanktonic and higher algae, as well as some invertebrates have been shown to contain antibacterial, antifungal and antiviral activities. The extensive literature on this subject has been reviewed^{2,3,5}. Some seasonal variation has been recorded. This would imply that the physical and chemical conditions of the microenvironment are critical for the expression of antibiotic activity by these organisms—a conclusion consistent with our extensive knowledge of the culture conditions necessary for the production of classical antibiotics such as penicillin.

In a study of the antibiotic activity of microorganisms isolated from various depths of the oceans (0–3,500 m), Krasil'nikova⁶ found that 124 of the 326 cultures collected were active against the test organisms *Staphylococcus aureus*, *Escherichia coli*, *Myobacterium luteum* and *Saccharomyces cerevisiae*. One actinomycete was also collected with a broad antibacterial spectrum. It is therefore not surprising that a variety of antibiotic activities have been observed in samples of filtered seawater³. At the same time relatively few studies have been taken to the point of isolation of the active principles involved. It is difficult to evaluate the relevance of most of these studies because the ratio of the minimum curative dose of the pure substance to the maximum tolerated dose for the infected animal is the first criterion used to determine the therapeutic potential of a substance with antibiotic activity. Moreover, in some cases, the active principle has turned out to be mainly of ecological significance, such as acrylic acid or brominated phenolic derivatives. One classical example of the potential of marine organisms has its origins in the observation that the culture fluid of a fungus, isolated from a sewage outfall into the sea, was toxic to a wide range of bacteria with an antibacterial spectrum similar to that of penicillin. Subsequently, Abraham, Newton and Florey showed that the fungus produced a number of antibiotics, notably a molecule containing a fused β -lactam-dehydrothiazine ring system (termed Cephalosporin C), as well as one with a fused β -lactam-thiazolidine ring system characteristic of the penicillin molecule⁷. Cephalosporin C and chemically-modified derivatives were toxic to a wide range of pathogenic bacteria, resistant to inactivation by the enzyme penicillin- β -lactamase and of low toxicity to man. Resistance to the inactivating enzyme was a very desirable property because a feature of therapy with penicillin was the development of strains of bacteria resistant to penicillin through selection of strains that produced the enzyme.

Other defined chemicals of marine origin with selectively toxic properties have been used in clinical medicine. Thelepin (a derivative of coumaran-2-spiro-cyclohexane, containing three bromine atoms per molecule) obtained from extracts of the polychete, *Thelepus setosus*, is an antifungal agent with a potency comparable to griseofulvin (a derivative of a coumaran-2-spiro-cyclohexane containing one chlorine atom per molecule), to which it bears a strong structural resemblance⁸. Kainic acid (2-carboxy,3-carboxymethyl,4-isopropenylpyrrolidine) is an efficient anthelmintic used in Japan to eliminate intestinal worms. Kainic acid is the active principle of the red algae, *Digenia simplex*⁹. Dried powders or syrups prepared from extracts of *Digenia* and other red algae have long been used as anthelmintics, with low toxicity to man, in Asia and in some countries bordering the Mediterranean⁹. The chemical nature and properties of antibiotics produced by marine organisms may well be the most rewarding area for definitive study in terms of human medicine. A programme of culture, screening, the selection of high-yielding strains and other development would require a considerable and broad-based effort, even if carried out in association with pharmaceutical companies who have experience with similar work involving terrestrial organisms. Apart from the speculative nature of such a venture, a purely mercenary aspect could limit support—as soon as the chemical structure of a drug is known an attempt can be made to make it available by

total chemical synthesis. While this can happen in some cases, most of our antibiotics such as the penicillins, streptomycin and tetracyclines are extremely complex structures and continue to be produced by the culture of high-yielding strains of microorganisms on a commercial scale.

Systemic drugs

There are about 2,000 venomous or poisonous species widely distributed among the marine fauna, with some concentration in tropical seas. The property of venom or poison biosynthesis is one mechanism that ensures species survival in densely populated ecosystems and represents a very successful form of chemical evolution and metabolic diversity from the common pattern. The general biology, chemistry and possible treatment of human illness caused by venom and by eating poison-laden fish, has been comprehensively reviewed by Halstead¹⁰. One of the most potent poisons known is tetrodotoxin (an amino perhydroquinazoline), a constituent of the liver and gonads of certain puffer fish (Tetraodon group)¹¹, porcupine fish (Diodontidae), ocean sunfish (Molidae), newts (Taricha) and some South American frogs. The flesh of the puffer fish is regarded as a great delicacy by Japanese gourmets, and a chef's reputation depends entirely on his ability to remove every trace of the toxic organs from the fish, and on the number of his guests who remain alive after eating his food. The dramatic and toxic effects of sampling sunfish roe is recorded by Captain Cook in his diary for 1774 (ref. 31). A lethal dose to man is about 10 µg per kg body weight and tetrodotoxin is about 160,000 times more potent in blocking axonal conduction than cocaine¹². It is extensively used as an inhibitor in neurophysiological research since it has a unique action on the gating of sodium channels or holes in excitable membranes. At low dosage levels, tetrodotoxin has been used clinically as a muscle relaxant and a pain killer in neurogenic leprosy and in terminal cancer³. It has also potential as a local anaesthetic¹³. The conclusion that tetrodotoxin is an endogenous fish poison¹¹ requires direct proof. Saxitoxin (a trialkyl tetrahydropurine derivative), is produced by strains of dinoflagellates of the genus *Gonyaulax* and has very similar but not identical pharmacological properties¹⁴. A massive overgrowth of these dinoflagellates and other phytoplankton can occur at certain times of the year in conditions that are not fully understood. This overgrowth develops to the point where the sea becomes discoloured and is termed a red-tide phenomenon. The bloom of phytoplankton can result in mass mortalities of fish due to toxins or to oxygen deprivation in the area of the bloom—but not of shellfish that consume the organisms and accumulate the toxin. This food-chain mechanism is presumably responsible for the periodic outbreaks of mussel poisoning or paralytic shellfish poisoning of man that have been recorded in recent years and in historical records¹⁴. It remains to be established whether saxitoxin is produced together with other toxins by the genus *Gymnodinium* which is also associated with red-tide phenomena. Apart from the venoms and poisons there are many reports that extracts of marine organisms have pharmacological effects on mammalian and other tissue preparations which can be described in such terms as anti-tumour, hallucogenic, neuroactive, cardioactive and cardiovascular^{2,3,5}. In some cases, the active principle has been identified. Interest in the ability of several genera of octopus to paralyse their prey by means of secretions from the posterior salivary glands led to the isolation of the peptide with the linear sequence, Pyroglu-Pro-Ser-Lys-Asp-Ala-Phe-Ile-Gly-Leu-Met-NH₂. This peptide from the salivary gland of *Eledone moschata* was termed eledoisin¹⁵, and proved to be a highly potent hypotensive agent in dogs—at least 50 times as active as histamine, acetylcholine and bradykinin on an equivalent

basis. Eledoisin and the related hexapeptide, Lys-Phe-Ile-Gly-Leu-Met-NH₂ (ref. 16), also stimulate extravascular smooth muscle and effect an atropine-resistant increase in salivary secretions. A cardioactive chemical, possibly an aromatic amine²² has been isolated in crystalline form from the branchial heart tissue of the primitive hagfish, *Eptatretus stoutii*¹⁷. The amine is a potent stimulator and pacemaker of the mammalian heart. This observation may be related to the fact that the heart of the hagfish consists of four pulsatile organs that operate independently—the systemic or branchial organ being aneural.

The possibility that some of the unusual metabolic products of marine organisms would have a selectively toxic action on tumour tissues in mammals remains an interesting possibility. Several have been identified, such as mercenene, a glycopeptide present in the common clam, *Mercenaria mercenaria*, during the summer months which is apparently non-toxic to mice at therapeutic levels¹⁸, and ill-defined substances present in the tentacles of two topical polychaetes¹⁹. The latter study would partly sustain the claims of folklore that cooked tentacles of *Lanice conchilega* had beneficial effects on certain forms of human cancer. One potent clinical anti-tumour agent has its origins in the discovery by Bergmann in 1968 that the West Indian sponge, *Tethya crypta*, contained large amounts of unusual arbinosyl-nucleosides. This led to the chemical synthesis and testing of a variety of nucleosides containing the unusual sugar moiety²⁰. 1-β-D-arabinofuranosyl cytosine (Ara-C) is a potent inhibitor of mammalian cell growth and is used extensively in chemotherapy in cancers such as leukaemias^{21,22}. Ara-C is known to be slowly phosphorylated to the 5'-triphosphate and has been shown to be incorporated into RNA and DNA and to inhibit DNA synthesis. Recently, a liponucleotide analogue, arabinofuranosylcytosine-5-diphosphate-1,2-dipalmitin has been synthesised and shown to be a potent inhibitor of leukemic cells (strain L 5178Y) in mice²³. It is claimed that cellular metabolism of the liponucleotide would generate Ara-CMP and hence the 5'-triphosphate at a faster rate than that resulting from direct phosphorylation of Ara-C by tissue kinases.

Attention has so far been concentrated on the nature and possible functions of the products of metabolic diversity and cellular specialisation in poikilothermic (cold blooded) marine organisms. Many patterns of metabolism are, however, common to all forms of life and certain hormones and other agents of metabolic control have a potential role in certain human disease states. In most cases these substances have been obtained from lower vertebrates sharing the same ultimate primitive ancestors as the mammals. One example was the extensive use of fish insulin, isolated mainly from the discrete islets of Langerhans of the tuna fish, to treat diabetics in Japan in the period 1939–45 (ref. 24). Other fish insulins, such as that of the cod *Gadus callarias*, have essentially the same specific hormonal activity in mammals as that of the homologous insulin. About 40% of the amino acid residues in cod insulin differ from those of bovine or porcine insulin but the invariant residues and features necessary for hormonal activity are conserved²⁵. In contrast, it was shown by Copp and others that the peptide hormone calcitonin, isolated from the ultimobranchial glands of the salmon (*Oncorhynchus*), was about 100–200-fold more active in man than the corresponding porcine hormone. This dramatic difference in activity has now been attributed to the greater resistance of the salmon hormone to degradation in the circulation of the mammal²⁶. A rather surprising development in recent years has been the discovery of relatively large amounts of prostaglandins in strains of the West Indian gorgonian, *Plexaura homomalla*²⁷, and in other marine organisms²⁸. The prostaglandins and thromboxanes constitute a family of related compounds that can be regarded as derivatives of the twenty-carbon atom monocyclic fatty acid, prostanoic acid.

These compounds were originally identified in mammalian tissue by Bergström in 1967 and have subsequently been implicated as the regulators of a very wide range of critical life processes in mammals, and in some disease states. Only trace amounts occur in the blood and tissues, both the prostaglandins and the thromboxanes, being formed by controlled biosynthesis from essential fatty acids through the intermediate prostaglandin endoperoxide. For this reason the gorgonians may well prove to be a useful starting material for chemical modification of prostaglandins to the mammalian types. This route may be a useful alternative to the successful but more complex process of total synthesis. An interesting feedback to marine biology is some evidence that the induction of prostaglandin synthesis (by hydrogen peroxide) is responsible for synchronous spawning in both male and female molluscs²⁹. It is thus tempting to speculate that the chemical signal effecting epidemic or synchronous spawning in the natural environment may be the concentration of oxidising free radicals generated by the light-induced photolysis of water.

Postscript

The only integrated proposals for the definitive study of a selection from the vast number of biologically active substances of marine origin have been oriented towards human medicine. A report to the Congress of the United States³⁰, contained a recommendation that a National Institute of Marine Medicine and Pharmacology should be established

as one of the National Institutes of Health. This recommendation has not so far been implemented, but a pharmaceutical company has recently opened a new laboratory in New South Wales, Australia—the Roche Research Institute of Marine Pharmacology.

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Bioluminescence of marine organisms

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Luminescence is a feature common to many marine organisms. Certain squid and fish utilise luminous bacteria as symbiotic sources of light, and the symbionts have physiological features which are of potential advantage in the association. The luciferins of some marine fishes, squids, crustaceans and coelenterates are of very similar chemical structure, though differing markedly from the luciferins of non-marine forms. Luminescence in many marine animals is probably used for ventral camouflage purposes.

THOSE whose lives have been closely associated with the sea and its resources have long been aware of both its general luminescence and that of many of its inhabitants. The first written record concerning luminescence of the sea has been attributed to Anaximenes (about 500 BC) and accounts of luminous marine animals have been available at least since the younger Pliny described the luminescence of *Pholas* and medusae¹. Bioluminescence has always been a source of wonder, often of awe, accentuated in earlier years by the conceptual difficulties of separating the phenomenon of light from that of heat. Indeed there is still occasional (unfounded) alarm that luminescent 'sparks' may trigger off explosions in oil tankers. By the mid-nineteenth century a variety of deep water marine animals had been taken which bore structures variously described as pores, slime glands or accessory eyes. It only became apparent that these

organs were for the production of light, rather than its perception, when the naturalists aboard HMS Challenger observed the emission of light from the ventral photophores of euphausiids and hatchetfishes². Only in this last century has the nature and occurrence of bioluminescence begun to be elucidated. Taxonomists have even occasionally enshrined the real (or sometimes imagined!) luminous abilities of many marine organisms in their names. Examples include *Photobacterium phosphoreum* (bacterium) *Pyrocystis* (dinoflagellate) *Pelagia noctiluca* (medusa) *Euphausia*, *Pyrocypis*, *Metridia lucens* (crustaceans) *Watasenia scintillans*, *Symplectoteuthis luminosa* (squids) *Pyrosoma* (tunicate) *Lampanyctus*, *Photoblepharon* and *Benthosema* (fishes). Even in common parlance lantern-fishes and fireworms provide marine counterparts to lighting bugs and fireflies.

This review deals briefly with three aspects of marine luminescence, namely the symbiotic luminous bacteria, the comparative chemistry of marine systems, and the use of luminescence as a camouflage.

Symbiotic luminous bacteria

All luminous bacteria require marine or other saline conditions, and they are present at all depths in the ocean, albeit in relatively small numbers. They can be grown in mass cultures in the laboratory and consequently more is known about the chemistry and molecular control of their luminescent system than that of almost any other organism^{3,4}. The luminescence of bacteria is normally continuous, with maximum intensity corresponding to a similar

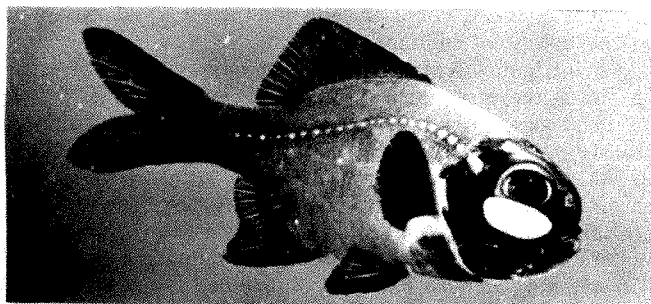


Fig. 1 *Photoblepharon palpebratus*—the large light organ below the eye contains luminous bacteria.

maximum in luciferase synthesis. The initiation of luciferase synthesis is dependent on the accumulation of an auto-inducer in the culture medium; very dilute cultures do not luminesce⁴. Some cephalopods and many fishes have taken advantage of the availability of luminous marine bacteria and developed organs in which a population of these microorganisms is maintained, thereby providing the host with a symbiotic source of light. Two genera of luminous bacteria, *Photobacterium* and *Beneckea*, are currently recognised⁵. It seems that *Beneckea* includes only free-living strains, whereas all symbiotic strains should be included in *Photobacterium*⁵. It has recently been shown that the synthesis of the luminous system of some species of *Photobacterium* is enhanced at growth-limiting low oxygen concentrations⁶. This has obvious ecological significance in the symbiont's environment, as do other features of its physiology⁷, and is not the case in *Beneckea*⁸.

Some 60 yr ago enthusiasm for symbiotic bacteria as the source of animal light reached such a pitch that some investigators held it likely that all luminous organs contained symbionts. Symbiotic bacteria are now recognised as responsible for the luminescence of certain loliginid and sepiolid squids, and a considerable number of fishes. Most coastal luminous fishes utilise bacteria (Leiognathidae, Eretmophoridae, Acropomatidae, Steindachneriidae, Trachichthyidae, Monocentridae, Anomalopidae and some Apogonidae⁹). Bacterial systems are, however, also present in oceanic fishes at all depths, namely in the benthopelagic Macrouridae⁹, the mesopelagic Opisthoproctidae^{10,11} and the bathypelagic anglerfishes^{12,13}. In most fishes the luminous bacteria are associated with one or two gut diverticula. In the Anomalopidae and Monocentridae, however, each fish has a pair of bacterial organs, just beneath the eyes in the former (Fig. 1) and on the lower jaw in the latter. The other exceptions are the anglerfishes, in which bacteria are maintained in the esca, a luminous bulb placed at the tip of a modified dorsal fin ray. In the anglerfishes *Ceratias* and *Cryptopsaras* two or three more posterior fin rays are also modified into bacterial bulbs (the caruncles)¹³. All bacterial organs open to the exterior, either directly through a pore or through the gut lumen (Table 1). Although luminous bacteria from gut-associated organs are generally easy to culture on artificial media, self-luminous cultures have not yet been routinely obtained from either the Anomalopidae or the anglerfishes. Starvation of *Anomalops* leads to a gradual dimming and final extinction of its luminescence, correlated with a great reduction in the number of bacteria in the light organs¹⁴.

Animals utilising bacteria have particular problems with which to contend. The bacteria must be restricted to the particular organ, and not allowed to spread indiscriminately (luminous sand-hoppers suffering from a general uncontrolled infection of luminous bacteria are occasionally reported¹⁵). The culture must be maintained in good condition and without contamination by other bacteria. Some symbiotic strains produce antibiotic substances which may

inhibit contaminants¹⁶. Surplus or dead bacteria must also be removed. Most importantly the light organs of successive generations must be reinfected with the appropriate strain. While this presents little difficulty in gut-associated systems, the mechanisms used by other fishes, particularly the anglerfishes, are quite unknown. This may perhaps be a contributory reason for the very small number of light organs present in animals utilising bacteria. Bacteria luminesce continually, and some means of controlling the light emission from the organ is therefore necessary. This may be effected by obscuring it with chromatophores¹⁷, rotating the organ, occluding it with a shutter^{18,19} or by restricting its blood supply¹³.

In non-bacterial or intrinsic luminescent systems different organs tend to be used for different purposes. The very limited number of light organs present in animals using bacteria has resulted in a remarkable behavioural versatility in their use; the same organ may be used for prey illumination, escape from predators, and both schooling and sexual communication²⁰.

Comparative biochemistry of marine luminescence

The discovery in the seventeenth century of inorganic chemiluminescent systems, or 'phosphori', overlapped Boyle's classical investigations of the luminescence of rotting wood and meat, and for a long period subsequently all natural luminescence was generically ascribed to a 'phosphorus'. As late as the nineteenth century when Edward Forbes persuaded his friend George Wilson, the eminent chemist, to investigate the luminescence of the sea-pen *Pennatula*, Wilson concluded: "On the whole I believe it most probable that the animal secretes a spontaneously inflammable substance. It may be a compound of phosphorus, but it is not necessary to assume that it is"²¹. Organic chemiluminescent compounds were only discovered in the latter part of the nineteenth century, and were a necessary prelude to the understanding of the chemical nature of bioluminescence.

In 1887 Dubois demonstrated the luciferin-luciferase reaction on material from the mollusc *Pholas*²¹. It was subsequently found that this general reaction could be demonstrated in only a minority of species tested²². This led to the conclusion that a very large number of quite separate chemical systems had been evolved in different species. Even now the chemistry of very few luminous systems is understood in any detail^{23,24}. This to a considerable extent reflects the difficulties of obtaining adequate quantities of active material for investigation. For example, some 10,000 specimens of the medusa *Aequorea* were needed to produce 5 mg of the active protein²⁵, and 10,000 specimens of the squid *Watasenia* were also used in the initial isolation of its luciferin²⁶. Extracted chemical systems were, until very recently, apparently either of the luciferin-luciferase type or photoproteins. The latter are high molecular weight compounds which require no additional enzyme and emit light in the presence of oxygen and/or an

Table 1 Comparative features of bacterial and intrinsic light organs

Bacterial organs	Intrinsic organs
Restricted to a few squids and fishes	Widely distributed
Usually 1 or 2 organs (never > 4) per species	Often numerous (several thousand in some fishes)
Only one type in each species	May be several types in each species
Always open to the exterior	Often closed
Often associated with the gut	Not usually associated with the gut

appropriate trigger²⁴. In the case of aequorin, the most studied photoprotein²⁷, only calcium ions are necessary, and light emission will occur in the absence of oxygen. In other photoproteins, such as that from the polychaete *Chaetopterus*, molecular oxygen and other cofactors are necessary²⁸. The demonstration that either a luciferin-luciferase system or a calcium-triggered protein can be extracted from the sea pansy *Renilla*^{29,30}, depending on the methods used, and the fact that the 'chromophore' of aequorin³¹ is chemically very similar, if not identical, to *Renilla* luciferin³², has led to a reappraisal of the photoprotein concept³³. It now seems that photoproteins may be regarded as stable luciferin-luciferase complexes. If this complex remains stable when oxygenated then a 'pre-charged'³⁴ photoprotein such as aequorin is obtained³⁵. In general it is likely that the oxidation pathway proceeds through the formation of either a dioxetane^{3,33} or a linear peroxide³⁶.

The structure of the luciferin of the ostracod *Cypridina* was one of the first to be elucidated. It is an imidazopyrazine³⁷, and in recent years strikingly similar chemical structures have been found in a number of other marine luciferins. The luciferin of *Renilla* (and the chromophore of *Aequorea*) is a similar compound, as is that of the squid *Watasenia*³⁸, and the decapod shrimps *Oplophorus* and *Heterocarpus*^{39,40}. It is clear that these luciferins can be produced by the cyclisation of a simple tripeptide (composed of tryptophan, isoleucine and arginine in *Cypridina*), and a number of active model compounds have been synthesised using different combinations of amino acids⁴⁰.

A luciferin apparently identical to that of *Cypridina* has been found in a number of fishes including *Parapriacanthus*⁴¹, *Apogon*⁴², the midshipman *Porichthys*⁴³ and the lanternfish *Diaphus*⁴⁴. Ostracods were found in the gut of some *Parapriacanthus* specimens and as the fish stores its luciferin in gut diverticula it was suggested that the luciferin might be obtained from its crustacean food^{45,46}. Some support for this conjecture has come from recent work on *Porichthys notatus*. Californian populations of this fish are luminous, but Puget Sound populations are not—although they possess a full complement of photophores. They lack luciferin, but produce luciferase normally. When fed *Cypridina*, or injected with natural or synthetic *Cypridina* luciferin, they too luminesce normally⁴⁷⁻⁴⁹. This remarkable situation suggests the need for an exogenous source of luciferin for normal luminescence, or at least for its induction.

There is clearly a common link in the chemical nature of the luciferins of a number of taxonomically unrelated marine animals. Despite the fact that the luciferins of fireflies⁵⁰, the mollusc *Latia*⁵¹, the oligochaete *Diplocardia*⁵² and luminous bacteria have very different structures, it is nevertheless remarkable that luciferins with very similar structures have been obtained from four different marine phyla. It will be of great interest in the future to determine whether other marine species, many of whose systems have a superficial biochemistry rather different to that of *Cypridina* or *Renilla*, utilise luciferins of similar general structure.

The colour of the emitted light may be determined in a number of ways⁵³. Pioneer work on insects⁵⁴ and ostracods⁵⁵ showed that the colour of the light emitted during the oxidation of a luciferin is determined by the source of the luciferase. Changes in the molecular environment of the luciferin, whether brought about by attachment to the luciferase or by physical factors such as heat, cations or pH, can produce different emission wavelengths. Work with model luciferins of the *Renilla* type has shown that different degrees of ionisation, in turn partly dependent upon the particular tripeptide used, can give rise to very different emission maxima. It is therefore theoretically possible that different marine luciferins, derived from dif-

ferent tripeptides, could account for the observed variations in the colour of bioluminescence⁵³.

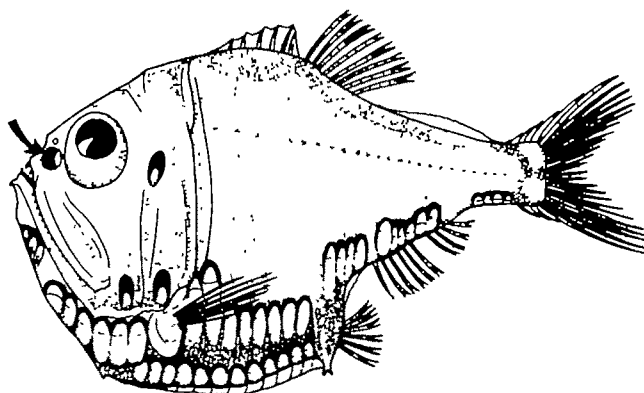
An intriguing feature of the luminous systems of some species is the presence of secondary emitters in the tissues. In these cases the energy of the luminescent reaction is transferred to a fluorescent material which then emits light at its own characteristic wavelength. Both *Renilla* and *Aequorea* contain a green fluorescent protein associated with the luminous tissue. *In vivo* light is green ($\lambda_{\max}$ 509 nm) but if the green fluorescent protein is removed a blue ($\lambda_{\max}$ 470–490 nm) *in vitro* emission is obtained. Addition of the green fluorescer to the *in vitro* system produces a green emission once more. Species lacking the green fluorescer (for example *Pelagia*, *Mnemiopsis*) emit only blue light both *in vivo* and *in vitro*⁵⁶⁻⁵⁸. Energy transfer has also been implicated in some fish⁵⁹ and squid⁶⁰ and may be responsible for bimodal emission spectra. It is tempting to consider that the red fluorescent proteins extracted from within the red-emitting photophores of the deep-sea fishes *Pachystomias Malacosteus* and *Aristostomias* may be involved in a similar process (P.J.H., unpublished).

Ventral camouflage

During the deep-sea expeditions of the late nineteenth and early twentieth centuries it became increasingly obvious that a great many deep sea animals bore light organs which were predominantly ventrally directed (Figs 2, 3 and 4). This is the case both in decapod crustaceans and in cephalopods, but is most prominently developed in fishes, both cartilaginous and bony. In 1916 Dahlgren, discussing cephalopods, noted "any animal situated below such a squid and looking upward at it from below would see a bluish light that would blend with the sunlight"⁶¹. Rauther applied the same hypothesis to fish⁶², but it was not until the idea of ventral camouflage was dealt with in detail by Fraser⁶³ and Clarke⁶⁴ that it achieved wide acceptance. In essence, animals living at depths still subject to background illumination from the surface are believed to illuminate their ventral surfaces so as to match the background, and eliminate the silhouette which would otherwise expose them to a predator from below. The use of this concept of ventral camouflage, or counter-illumination, was considered during World War 2 as a method of camouflaging planes during daylight sorties. Lights were to be fixed to their undersides to reduce or eliminate their silhouette, but the power requirement ruled out the idea at an early stage.

Recent work has been aimed at providing experimental support for this attractive hypothesis, but much of the

Fig. 2 The hatchetfish *Argyrops aculeatus*. The photophores are directed ventrally, with the exception of the preorbital organ (arrowed) which shines into the eye. This organ may act as a reference standard for comparison with ambient light.



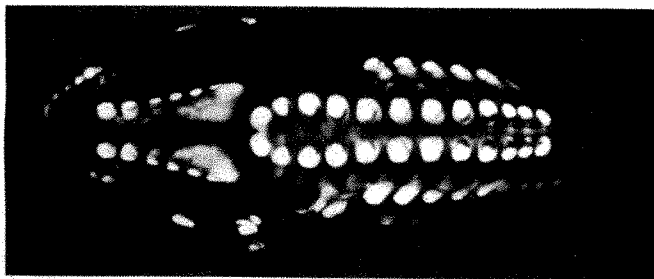
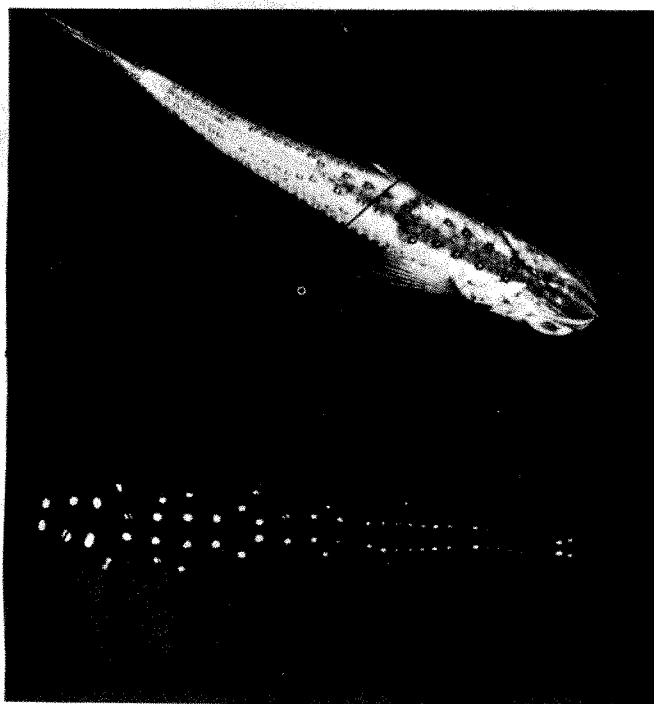


Fig. 3 Ventral view of a luminescing *Argyropelecus olfersi*.

evidence is still circumstantial. Counter-illumination provides a rationale for the otherwise anomalous positions of numerous photophores. Many otherwise transparent squids have photophores beneath the eyes and ink sac, their only opaque organs. Many crustaceans also have photophores beneath the eyes. In decapods such as *Oplophorus* the photophores on the pereopods will contribute to the ventral illumination of the body when these limbs are folded in a swimming position. Ventral camouflage provides an explanation for the extraordinary anatomy of the mesopelagic fish *Opisthoproctus* (Figs 5, 6). The light from its single bacterial rectal organ is spread over the whole flattened sole of the fish by an elaborate light guide system^{10,11}. Similar anatomical diffusion of the light over the ventral surface is found in many other fishes utilising bacteria. The restricted number of light organs in such animals requires that elaborate accessory optical structures be present if a general ventral illumination is to be achieved^{17,64}.

Detailed anatomical and experimental studies of hatchetfish photophores have shown that their reflecting systems are so arranged that the angular distribution of the emitted light almost exactly matches that of the ambient downwelling daylight (Fig. 7)⁶⁵⁻⁶⁷. This arrangement ensures that the ventral camouflage is equally effective when observed from almost any angle. Hatchetfishes have a filter pigment, with a transmission maximum at about 480 nm, in the aperture of their photophores⁶⁸. Similar filter pigments are

Fig. 4 A lanternfish (*Myctophum* sp.) showing the ventral array of photophores (a) and its appearance when luminescing (b).



found in many other fishes, squids and crustaceans (Fig. 8). Measurements of the emission spectra of the photophores of certain fish before and after the removal of the filter pigments, have shown that the effect of the filter is to modify the emission spectrum so that it finally closely approximates that of the down-welling daylight (Denton, E.J., and Herring, P.J., unpublished). Some animals, at least, are therefore able to match both the angular and spectral distribution of ambient daylight. Such a system will obviously be most effective if the fish remains in a constant light intensity. It has indeed been demonstrated that some animals, in the form of scattering layers, remain very close to particular isolumens⁶⁹. Changes in ambient intensity also affect the depth distribution of many species, not only by day but also by night⁷⁰. If, on the other hand, an animal can alter the intensity of its own bioluminescence it achieves much greater behavioural flexibility, as its movements in the water column can then become independent

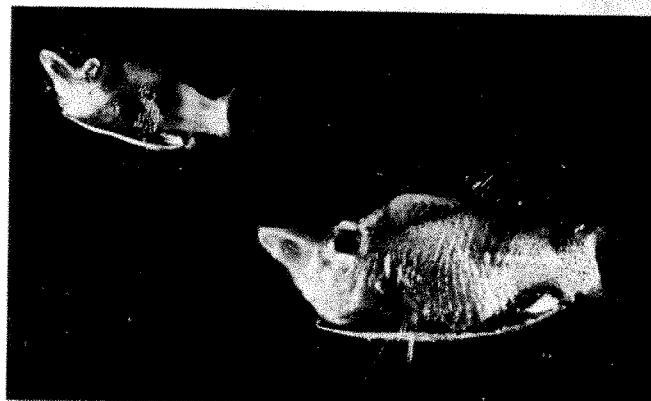


Fig. 5 Two specimens of the mesopelagic fish *Opisthoproctus grimaldii*. Luminous bacteria are maintained in a rectal diverticulum.

of diurnal changes in ambient light. Experimental evidence is now accumulating which indicates that in some animals at least the intensity of ventral bioluminescence can be varied. Free-swimming squid^{71,91}, ponyfish⁷² and luminescent fishes^{73,74} have been observed to alter their ventral luminescent output in response to changes in overhead light.

By what means does the animal ensure that its bioluminescent output matches the ambient light intensity? Many stomiatoid fishes have an orbital photophore that shines into the eye. It has been suggested that these organs allow the eye to 'light adapt' so that the fish is not blinded by its own emission⁷⁵. It seems more likely, however, that the function of this organ is to act as a reference standard for comparison with the light from above⁷⁶. Certainly, in hatchetfishes at least, the intensity of the orbital photophore waxes and wanes in concert with that of the ventral organs⁷⁷, a necessary feature of such a hypothesis. The supraorbital photophore in lanternfishes such as *Tarletonbeania* may also serve this purpose⁷⁸, while extra-ocular photoreceptors, the photic vesicles, may be similarly utilised in squids⁷⁸. Reference systems cannot however be identified in many fishes and other animals with ventral photophores. There seems no means, for example, whereby the upwardly directed eye of *Opisthoproctus* can detect the downwardly directed luminescence of its sole. It is possible that in such cases a fixed intensity emission may be modified by the responses of overlying chromatophores, and these in turn respond directly or indirectly to ambient light intensities. The colour and pattern responses of many shallow water fishes have an analogous feedback control requirement to the ventral bioluminescence of deep-sea species.

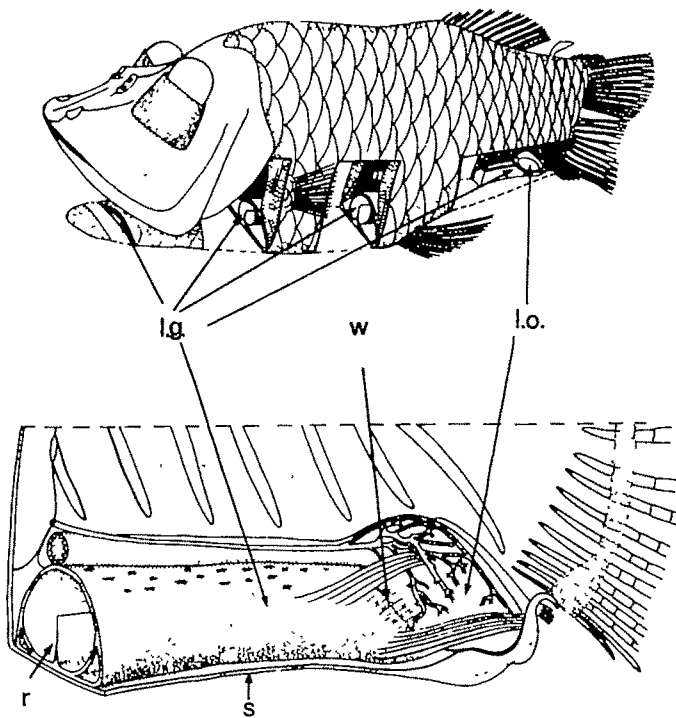
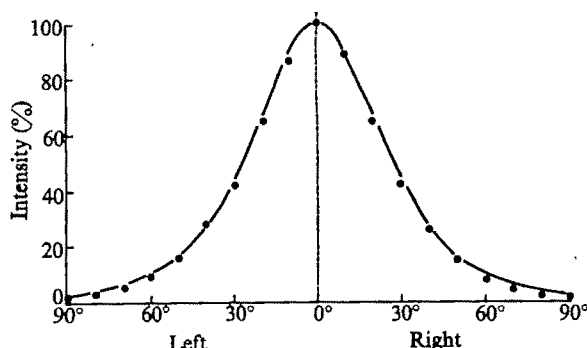


Fig. 6 The anatomy of the light organ of *Opisthoproctus soleatus* (from ref. 10). a, Lateral view showing the position of the light organ (l.o.) and light guide (l.g.). b, Detail of the light organ with its window (w) into the light guide, a hyaline tube surrounded by reflecting platelets (r) and diffusing the light over the whole area of the flattened sole (s).

Ventral counter-illumination can only be of camouflage value at depths in which downwelling light provides a significant background illumination, namely the epi- and mesopelagic zones²³. In the surface waters during the day the ambient intensity is too great to be countered by bioluminescence, and other aids to concealment, such as transparency, have to be used. Ventral illumination will be of greatest effect during the day in the mesopelagic zone, but at night will be equally effective much nearer the surface. The shallower living animals will need to emit more light than those in deeper and darker regions, and their respective ventral luminescent systems should give some indication of this. It was recognised very early that there is a correlation between the depth of a species and the development of its photophores²⁴. Murray and Hjort²⁵ noted that the fish *Cyclothone signata* had larger photophores than *C. microdon*, which was caught at greater depths. This is a

Fig. 7 A comparison between the angular distribution (in the transverse plane) of light from the hatchetfish *Argyrolepecus affinis* (●) and a computed curve for daylight in the sea (solid line) (from ref. 67).



general feature of many groups of animals, though it has only become clear with the advent of better techniques for the determination of the detailed vertical distribution of pelagic and planktonic organisms. Thus in *Cyclothone* the shallowest species *C. alba* and *C. braneri* have larger photophores than the deep *C. acclinidens* and *C. microdon*. The deepest species, *C. obscura*, has only rudimentary photophores. Similar series are found in species of *Gonostoma*, *Stomias*, the hatchetfishes, and the lanternfishes. In the latter group the photophores of the deeper genera (for example, *Lampadena*) are much smaller than those of shallower genera (for example, *Hygophum*, *Diaphus*). Similar traits occur in other groups. The deepest species of the euphausiid genus *Thysanopoda* have relatively much smaller photophores than shallower species, and in the

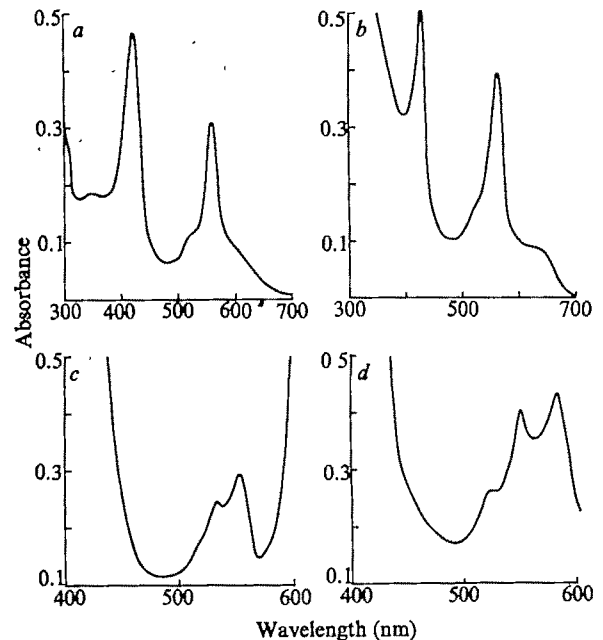


Fig. 8 Visible absorption spectra of pigments extracted from the photophore filters of four species of mesopelagic fishes. In each case the pigment is an effective blue filter, so that the final emission closely approximates the spectral distribution of ambient daylight in the sea. a, *Chauliodus sloani*; b, *Stomias boa*; c, *Valenciennellus tripunctulatus*; d, *Argyrolepecus aculeatus*.

shrimp genus *Systellaspis* the photophore size in different species also mirrors the depth distribution²¹. Some of the very deep living animals may use their photophores for camouflage only during their nocturnal migrations close to the surface.

Developmental information provides additional support. Many marine animals pass through a period as transparent larvae in the surface waters before sinking to greater depths as an adult. Those fish larvae that sink gradually during development tend also to develop their photophores gradually, with the result that they are appropriately equipped at all stages to mask their increasing opacity. Those that sink suddenly during a rapid metamorphosis tend to develop all their photophores at once, so that they, too, are equipped for their new light regime^{22,23}.

By no means all ventral photophores need be regarded as counter-illumination systems, and McAllister²⁴ has pointed out some of the problems inherent in any hypothesis of universal ventral camouflage. Many animals with ventral photophores are found close to the bottom during the day (for example ponyfishes). Counter-illumination would serve no purpose during the day—but might still be nearer the surface at night. Equally, if the orientation of of advantage in moonlit waters were the fish to migrate

a counter-illuminating animal changes in the water its camouflaging light could become a disastrous beacon. One of the stumbling blocks of a camouflage hypothesis is that submersible observations sometimes describe how many fishes seem almost randomly orientated in the water^{83,86}, though the animal may not necessarily be luminescing⁸⁷. Certain decapod and euphausiid shrimps can alter the orientation of their photophores^{88,89}, and this would be an effective means of compensating for changes in their orientation in the water. A further difficulty raised by bathysphere observations is the visibility of luminescing fishes. If their camouflage is effective they should not be visible. Yet Beebe⁹⁰ could "plainly see the lights of their (hatchetfishes) downwardly-directed photophores when the fish swam above me". These observations seem somewhat at variance with the camouflage concept of ventral luminescence. Nevertheless, there is now very considerable evidence, albeit still largely circumstantial, providing support for the hypothesis.

Observations of bioluminescence are always fascinating and often perplexing; their interpretations are even more so. Both observations and interpretations have a long history of suspicion, or outright disbelief. In the early eighteenth century a letter was received by the French Academy of Sciences from the people of Cadiz¹ "importing that . . . they had seen the whole sea shining with a clear light almost like a liquid phosphorus, . . . the seawater being carried away in bottles gave the same light in the dark; that some drops of it being let fall on the ground shone like sparks of fire, and that linnen dipt in this water became luminous." The Academy responded to this quite accurate description of dinoflagellate luminescence by retorting "The fact having been well examined, is found to be false, . . . The Academy think, they do as much service to the public, in disabusing them with regard to false wonders as in recounting to them the true." In which category would they have included the Puget Sound *Porichthys*, fishes with a complete battery of mainly ventral photophores—none of which work?

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A hundred years ago

RAINFALL OBSERVATIONS IN THE EAST OF FRANCE FROM 1763 TO 1870.—In the *Bulletin Hebdomadaire* of the Scientific Association of France, of the 10th instant, Prof. Raulin gives an interesting historical account of all the rainfall observations made during these 108 years anywhere in that section of France which is marked off by lines joining Givet on the Meuse, Lauterbourg on the Rhine, Belley near the Rhon, and Decize on the Loire, and which thus comprehends seven well-marked regions, viz., the plain of Alsace, the chain of the Vosges, the plateaux of Lorraine and Bourgogne, the plains of Champagne and Bresse, and finally the chain of the Jura mountains. During the past three years Prof. Raulin has been engaged collecting all available materials for a monograph on the rainfall of this part of Europe, which, judging from his great monographs of the rainfall of other sections of France and of the rainfall of Algeria, will doubtless take its place as a permanent contribution of very high value to meteorological science.

Marine research in China

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This paper is a personal travel report from a visit to the People's Republic of China in September and October 1976. Oceanographic research, as seen from visits to institutes, participation in fieldwork and personal contacts, is discussed, with some emphasis on the physical and chemical aspects. There is a tangible striving for utility in research policy. Research activities are also to a large extent integrated with other functions of society. Marine research is of increasing importance in China today; there is a growing cognisance of the hazards involved in pollution of the marine environment. The technical standard varies; some institutes are equipped with older apparatus based on manual methods, whereas others had access to modern analytical instruments. Information about the important centres for oceanographic research, at Tsingtao on the Yellow Sea coast and at Kwangchow for the south China Sea is presented.

THE sea is of great historical importance in Chinese society. It has been a temptation as an escape out of a land locked by mountains to the west. It has also carried enemies in warships to the long China coast.

The Chinese have long been eminent seafarers. The most renowned of them was Cheng Ho, alias San Pao (he is best known under the latter name in Asia and on the Southern islands). From the mouth of Yangtze Kiang he started enormous trade expeditions with 500-foot ships and more than 20,000 men in the beginning of the fifteenth century, that is before Diaz, Vasco da Gama, Columbus and Magellan. Cheng reached India, Persia and Ethiopia. On later voyages he visited Zanzibar to the East of Africa and Timor near Australia.

Later there was a decline in Chinese seafaring. Internal antagonism during the Ming dynasty led to a prohibition of long voyages and all ships with more than two masts were broken up. The Chinese command of the sea was thereby irretrievably lost. The Portuguese and the Dutch soon became the new commanders of the seas. Later there followed a period of lively sea traffic to China (but without Chinese participation), and during the following centuries parts of the Chinese coast were colonised. Not until this century with the liquidation of most of the colonies (some of them, for example Hong Kong, are still left) has a new Chinese merchant navy grown up. Today this merchant navy is of a considerable size, often sailing under the flag of convenience of Somalia or Panama. Local river navigation has always been important and the transport of goods by junks has been the core of the Chinese transportation network.

The sea has long been a source of nutriment for the Chinese. The profession of the fisherman is mentioned in the oldest documents. Even more characteristic of the old Chinese knowledge of the sea is the use of marine algae for food. Algae are collected at sea and included in the fare where they are either eaten directly or cooked. Some

are preserved or dried and stored for later use. More than a hundred species are used for food.

Supply of resources from the sea

Interest in the sea is today focused on natural resources. The question, what can the sea give up in the way of food, minerals and energy? is being raised all over the world. When it comes to extraction of food from the sea we are today departing from the hunting stage where we catch whatever we can, to the farming stage where we plan and breed aiming at maximal yield but with protective measures taken to support the regrowth. In this field China has a great advantage in the knowledge that is accumulated from freshwater farming. For many thousands of years the Chinese people have collected information about fish breeding in ponds, of how to master the diseases of fish and of how to best feed and catch. Some of this knowledge can be transferred to conditions in the sea. Regarding

Fig. 1 Sketch map of China. The coastline is approximately 10,000 km long. Tsingtao on the Shangtung peninsula is a centre for marine research. Large mussel farms are kept in the shallow water of the Yellow Sea outside the town and marine algae are cultivated on beds in the tidal zone close to the shore. The Pohai Sea and Yellow Sea are steadily silting up (Shangtung was formerly an island and Peking was situated at the sea) due to mud deposits from the northern rivers. Kwangchow has several marine institutes and is now a centre for present research activities in the South China Sea.



minerals and energy, China, like many other countries, is now aware of the potential richness of the sea. Sea areas which were previously fairly uninteresting become suddenly valuable and territorial demands are raised and fishing rights are claimed.

Marine research

In these circumstances it is natural that marine research in China is expanding. Research institutes are growing, new vessels are built, field stations are established and new problem areas are investigated. My journey to China with visits to various marine institutes was arranged to study marine research and to get a view of its status today. It was a research exchange between China and Sweden and a group of Chinese scientists had the opportunity to study the corresponding Swedish conditions.

A comparison of research policies shows difference of principles between China and the 'Western countries'. In the West we have an extensive basic research and a parallel applied research which sometimes, when needed, uses results from the basic research. This basic research is sometimes termed free research and the applied research is called

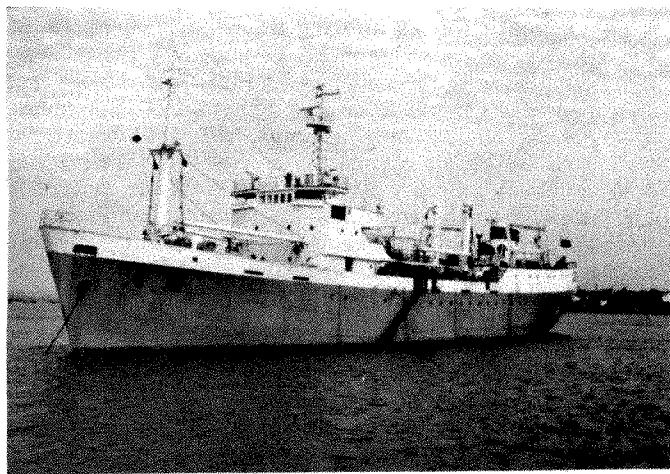


Fig. 2 The research vessel, The Red Star, anchored on the Pearl River near Kwangchow (Canton): 66 m length, 11 m width, 3.7 m depth, 1500 tons dead weight, 12 knots. It takes 100 tons of freshwater and 120 tons of fuel allowing expeditions of up to 3 weeks at sea with a crew of 35 (sailors and scientists). Many of the cruises are directed to the South China Sea. Home harbour is Kwangchow.

technical research. Free research is based on personal interests of individual scientists who find a certain problem area interesting. These scientists apply for finance from the scientific boards directing the research, are supplied with grants and make an investigation resulting in a report. In this way knowledge of previously unknown conditions is documented to form a field of knowledge where the gaps, one after the other, are filled in. The individual scientist at the time of the investigation may not necessarily see of what direct use the knowledge he accumulates can be. It is known empirically that basic research is profitable by creating a base for technical development, and the scientist can assume that the knowledge might come to use some time in the future.

This so called free research is not emphasised in China. Instead, research is strongly directed at problems. The investigation is expected to give a practical result as soon as possible and is therefore more like our technical research. The reason for this probably lies in Chinese modern history,

where the overall dominating aim was to free the population of over 800 million from starvation (a task carried out within one human generation). In this situation all marine research was aimed at an increase in the yield of nutriment from the sea, and it is possible that increased basic research will return with increased economic margins. It is probable that a weak basic research in the long run will check the applied research. On the other hand, the question can be raised as to whether we in the Western countries sometimes solve hypothetical problems that consume resources but are of no practical importance. Probably we have a lot to learn from each other. If we learn and take advantage of the pronounced utility in the Chinese research and the broad base for technology provided by the Western research, we should find a good formula for truly effective research.

A distinct impression from the collaboration with the Chinese scientists is that they are well aware of the events and progress of science in the West, but that they carefully sort out and make use of only the results that in some way are fit and good for China. Many of the senior scientists have, after the liberation, returned from institutions in USA and Europe. They master foreign languages, have a good overview of the research situation in the Western countries and today they often have leading positions in their institutes. The flow of information from Western literature is sufficient and the institution libraries often have the later important works in the field of marine research. Yet there are no signs of Americanisation or strong European influence in research policy and methods. As a visitor one gets, after a while, a strange feeling of being 'looked through'; one realises that the Chinese colleagues fairly well know one's situation without being interested in taking it over.

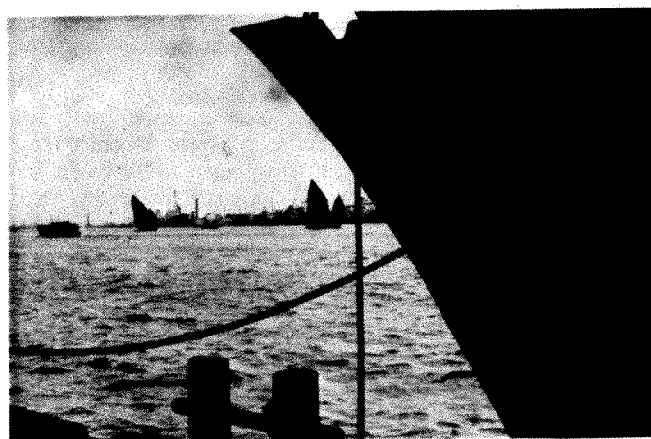


Fig. 3 Shanghai harbour. To the right the fore-end of a modern trawler. The signs mean Shanghai Fishing Company and are followed by a number. In the background modern trade ships and in front of them traditional wooden sailing junks carrying goods up the Yangtze Kiang river.

There is also an interesting difference regarding research means and methods. We can, in our countries, hardly purge ourselves from new ideas and methods which are pushing on, regardless of if we really should prefer them or not, whereas the Chinese society has found ways completely to control and direct outside influence.

There is a great interest in China throughout the world. Academia Sinica who mediate the scientific visits receive many applications from groups wanting to visit China, and they strain off a considerable part. But there is today a great Chinese interest in Western methods, knowledge and experience in the areas of pollution studies and environmental protection.

Much of the equipment at the research institutes consists of simple, sometimes almost primitive aids, much apparatus is worn out and also the basic resources like buildings and fixtures are often old and worn. On the whole, the technical standard is quite low. This depends partly on an actual low standard, but partly also on the thinking based on optimisation and utility that is found everywhere in China; human and material resources should be used in a judicious way, overdone technology has no mission; all activities should aim at an improvement of the vital conditions for the people.



Fig. 4 Buoy systems carrying nets on which blue mussels grow in the mussel farms south of Tsingtao in the Yellow Sea. (Photograph by Hans Ackefors).

On the other hand there is no lack of resources 'when they are needed', that is, when a problem is considered urgent. If, say, an unknown disease were to threaten the huge marine cultivation farms on the coast, large resources of personnel, research vessels and modern instruments would be available to attack the problem. Laboratory instruments were sometimes amazingly modern in design and function. As an example there were Chinese manufactured scanning recording spectrophotometers and gas chromatographs with linear temperature programming and all types of interchangeable detectors including electron capture. The instruments had also advanced industrial design in their performance, which is an indication of a high technical level. These instruments were manufactured in Shanghai.

Chinese marine research has access to a number of research vessels making regular cruises at sea. The Yellow Sea, being a productive shallow water area on the shelf has traditionally seen the main part of the research activities with a centre in Tsingtao on the Shangtung peninsula. The East China Sea coast has some importance for fishing and cultivation of algae. Today, however, the interest is largely concentrated on the South China Sea. This is caused by the increased awareness of the fish resources in the area and the recent discovery of potential oil and mineral resources. Field stations have been established on previously unoccupied groups of islands and an intensive investigation programme concerned with the marine geology, hydrography and fishery biology of the area has started. Unfortunately, it was not possible to visit the most interesting research vessel, the Red Star, as the ship was on a cruise to the South China Sea. The photograph, Fig. 2, was taken by another Swedish visitor (Olof Tandberg); it shows the Red Star which is approximately 2,000 tons dead weight.

Information was also obtained on two newly built ocean-going research vessels of approximately 10,000 tons dead weight, which were at that time on expeditions in the Pacific. These ships are controlled directly by the central government in Peking. Information on these ships has also been given in the information journal *Peking Review*, published in English in several countries. The ships have equipment for marine geological investigations and laboratories for chemical analyses. There are also many smaller research vessels, many of which operate in connection with field stations on the coast. In addition, research work and practical work are to a large extent integrated in China, so that, for example, fishing boats take samples for later analysis at the research laboratories.

Visits to marine research institutes

Practical oceanographic research work was studied during visits to a number of research institutes along the coast. A certain formalism and conformity characterised all such visits. The visit is introduced with a quiet chat where everybody is drinking tea, sitting in a rectangular formation along three of the four walls of the room. The talk is, however, mainly directed by the group leader of the visitors and the head of the reception committee, and does not have the form of a general discussion.

During continued tea drinking the political objectives of the institute are then thoroughly described by the political representative of the reception committee. It is obvious that chairman Mao's thinking has had and still has an immense influence on all activities in China. All institutes visited lean back on his statements as directives for their work. Some political concepts often recur. One such concept is the 'trinity' which can refer to peasants, workers and soldiers, for example, in discussions of recruiting students to the universities, or it can sometimes denote other constellations such as workers, technicians and supervisors in discussions of a technical problem. The fact that an instrument is constructed by an engineer, the assembly is planned by a supervisor and performed by a worker is in our language convention understood; we know it, but we do not say it. In China, it is felt important to underline such facts, to mark a new approach, a difference from the past, and when displaying an instrument these three anonymous but equal originators are mentioned.

Another concept is 'open-door research' which underlines the cooperation between research and production. The scientist should not retire into splendid isolation when solving his problems but carry out his research in the industries cooperating with the workers and considering their practical viewpoints of the problem.

After the information on the political objectives follows an account of the scientific research program, given by a scientist of the institute. Thereafter follows a guided visit to the different laboratories to see the activities and equipment. There is an open atmosphere during these visits, questions are answered, everything is shown on request, improvisations and suggested changes of the program are acceded to as far as is reasonable. (This was also the case for the free time during the programme. There were no restrictions on the movements and activities of the visitors.)

Apparatus and technical equipment

Chinese research laboratories have different, not so strict, partitioning into disciplines compared with that which we are used to. Problems which we would have split up into, say, a biochemical and a marine geological part, may in China be solved at the same place. The partitioning of fields of activities and translations of laboratory names made in the following are therefore often approximate and difficult to bring close to reality.

The research vessel the Red Star (Fig. 2) has a common water sampling equipment, with deep-going winches and water samplers, instruments for observation and measurements of physical properties, tube and gram samplers for sediments and laboratory on board for chemical analysis. Part of the equipment is carried on board for every expedition, for example, the photometer. There is also a laboratory for treatment and conservation of biological samples.

The institutes visited all had laboratories for analysis of water samples which were brought back from sea. Shantung Institute for Oceanography had a modern salinometer, based on electric conductivity of the sea water, of high sensitivity ($S = \pm 0.001\%$). This institute manufactured for calibration purposes, standard sea water which was sealed into glass ampoules.

As mentioned above, some institutes were equipped with modern gas chromatographs and spectrophotometers. Access to mass spectrographic analysis was also provided, although we did not see an installation during the visits. At Shantung Institute for Aquatic Products a cold atomic absorption instrument for a.o. mercury determinations was demonstrated.

The sampling equipment shown at some institutes was of conventional types. Russian designed grab samplers for sediment were shown in Kwangchow, and common types of plankton nets were also to be seen.

The basic equipment of the laboratories such as centrifuges, drying ovens, microscopes, water thermostat baths and oscilloscopes was in the main similar to what we are used to in our laboratories for routine oceanographic analyses.

The carbon-14 technique for measurement of the primary phytoplankton production was known and was studied at Hupeh Hydrobiological Institute at Yangtze Kiang. It was not yet in use at sea, however, and the primary production was determined according to the oxygen method using light and dark bottles.

Remote sensing as a tool in marine research is as yet very little used in China. The scientific element of the country's own satellite program is (according to verbal communication) merely used for studying meteorological problems. It has been said that China is probably taking information from the LANDSAT-satellites, but comments on that were not received.

Pollution problems are receiving an increasing interest as a result of the increased pollution by industrialisation. The scientists are aware of the problems and anxious to learn from other countries and share their experiences of solutions to the problems. It is unlikely that they will allow a mercury and cadmium catastrophe like that in Japan.

It is admitted, however, that DDT is a serious problem and that even deaths have occurred. Intensive research in the area of biological control is going on in order to replace the chemical biocides with biological control methods. Instrumentation for routine analysis of heavy metals and for DDT was seen at many laboratories.

The Institutes

The Institutes carrying out oceanographic research were the following.

South China Sea Institute of Oceanography in Kwangchow (Canton), is administered by Academia Sinica. It specialises in the South China Sea and has laboratories specialising in hydrography and meteorology, marine geology, marine biology and hydrophysics.

The institute was founded at Chanchiang on the south coast but was moved to Kwangchow in 1973. This institute

is administering the research vessel, the Red Star. It has also been engaged in pollution studies. There are 340 employees, including those in field stations. A new building for the institute comprising six floors of a total of 9,000 m² for research and administration is under construction. Another research vessel of about 1,000 tons is being built this year.

Kwantung Institute for Aquatic Products in Kwangchow. The institute specialises on the South China Sea. The main task is to develop and utilise the resources of the South China Sea. It has about 500 employees, most of them stationed on field stations belonging to the institute. A new building is under construction for the mother institute in Kwangchow, which will contain a political office, an administrative office and six laboratories. The institute has several smaller research vessels. One branch of their activities is also to spread scientific information among the people.

Shanghai Institute for Aquatic Products in Shanghai. The Institute had, after the cultural revolution 1968, no research vessels of its own, but its scientists are working from fishing boats in cooperation with the fishermen and the fishing company (open-door research). About 200 are employed. The Institute has a laboratory for marine resources.

Tsingtao Institute of Oceanology in Tsingtao. The institute belongs to Academia Sinica. Their working area is the entire coast from Pohai to the South China Sea. About 500 persons are employed. They have three research vessels of 1,000, 400 and 300 tons dw respectively and laboratories for marine physics, marine geology, marine chemistry and oceanographic research instruments. The institute is also engaged in pollution studies. Research is to some extent concentrated on the Yellow Sea shelf area. Sediment movements are also studied.

Shantung Institute of Oceanography in Tsingtao. The institute is mainly an educational institution. There are departments of marine hydrology and meteorology, marine physics, marine chemistry, marine geology and marine biology. There are about 500 employees and the number of students varies during the year. There are 840 places for a 3-yr education for marine scientists. There are also about 1,000 students on short term courses. Education is also given by correspondence. The institute has stressed work on pollution and environmental problems. For field work and excursions there are a number of small motor-boats.

Shantung Provincial Institute for Marine Products in Tsingtao, with about 220 employees. Working area is the Pohai Sea and Yellow Sea. Emphasis is on the living resources. Research is on effects of industrial sewage water on the marine environment.

Direct reference to Chinese scientists, and further details of marine research in China may be obtained from the author on request.

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articles

Possible relation between optical and very long baseline interferometer radio observations of the QSO 3C 345

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Existing radio and optical data on the variability of the QSO 3C 345 are summarised. Interesting correlations between the polarisation in radio and optical bands are evident; moreover successive outbursts have similar polarisation. This suggests that all outbursts are triggered by activity in the same central object rather than being a consequence of unrelated explosions of supernovae or similar objects.

SEVERAL QSOs show an apparent superrelativistic expansion in the radio which often continues for several years along the same position angle. On one interpretation, the expansion must be guided by a relatively permanent structure, and I have, therefore, looked for other signs of this structure. During outbursts, the annual mean plane of polarisation of the optical radiation from 3C 345 aligns with the direction of the superrelativistic expansion: the magnetic field associated with the optical radiation is therefore perpendicular to the expansion. There is weaker evidence that in the radio the average intrinsic plane of polarisation is perpendicular to the expansion vector. These regularities give more support to the idea that a QSO has an ordered and relatively stable structure than that it is made up of an array of unrelated sources.

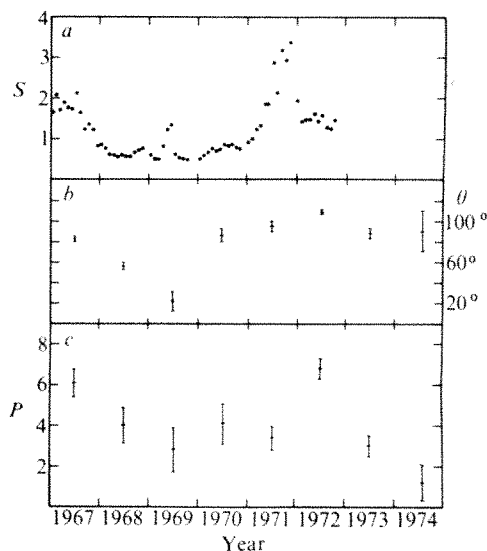
Superrelativistic expansion

3C 345 (redshift 0.595) is one of the three QSOs in which radio variability was first discovered by Dent¹. Its optical variability² was discovered soon afterwards, and also its associated property of a variable optical polarisation³. It has therefore been one of the most closely monitored sources at both optical and radio wavelengths. Recently, very long baseline interferometer (VLBI) radio observations at 2.8 cm by Cohen *et al.*⁴ and by Wittels *et al.*^{5,6} at 3.8 cm have shown that apparent superrelativistic expansion occurs in this source. Between 1974.15 and 1976.13, Cohen *et al.* found a separation increase of from 1.23 to 1.70 milliarc s on a two-component model. Wittels *et al.* found a separation increase of from 1.0 to 1.3 milliarc s from late 1971 to 1974 at the longer wavelength. In both cases, the position angle of the expansion was well defined with a position angle $105^\circ \pm 5^\circ$. Explanations of such expansion have been suggested in terms of (1) relativistic motion along the line of sight⁷, (2) a static array of sources whose flux variations simulate the expansion⁸, or (3) a model in which it is the phase motion of an actual expansion that

is observed^{9,10}. The second explanation requires that contractions should be observed as frequently as expansions, and this is not the case as far as is known. Sanders¹¹ has described a model of the third type in which the superrelativistic effect is produced by synchrotron emission from electrons travelling in a dipole magnetic field as seen from its equatorial plane. Each expansion originates from a relatively impulsive injection of relativistic electrons from a source near the centre of the dipole. The energy of these electrons is less than the energy of the magnetic field, and so the field survives, and the same expansion orientation may be expected for successive outbursts.

It seems likely that the impulsive injection of the electrons should be associated with an increase in the optical flux. A simple linear extrapolation back to zero component separation suggests that the present expansion originated between 1958 and 1963 (3.8-cm observations) or at about 1967 (2.8-cm observations). The time of origin is therefore quite ill-determined. Furthermore, if the beaming angle of the optical flux is very small (1–10 mrad) as suggested by Cocke and Pacholczyk¹², the actual optical emission associated with a specific radio expansion might

Fig. 1 Optical flux and linear polarisation properties of 3C 345: *a*, monthly means of the optical flux in mJy; *b*, annual means of the position angle θ of the polarised flux; and *c*, annual mean of the percentage polarisation.



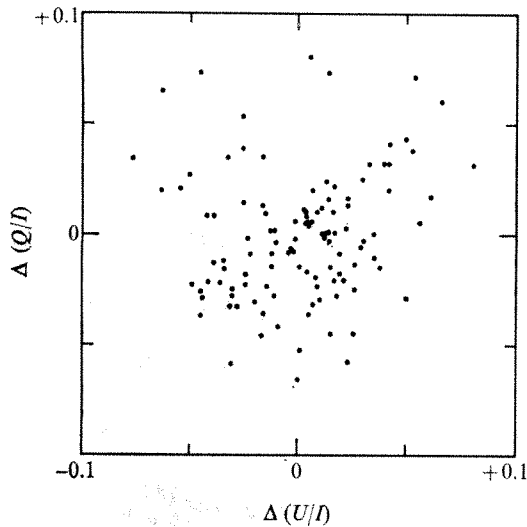


Fig. 2 The residuals of the normalised Stokes parameters for the optical flux from 3C 345 after removal of their annual mean values.

be missed if it did not occur in the direction of the Earth. But even in this case, if the model has a permanent magnetic structure, each optical event should carry in its linear polarisation the signature of the magnetic field geometry and this should be related to the geometry of the expansion.

Optical linear polarisation

Some 114 observations of the optical linear polarisation of 3C 345 have been made between 1967 and 1974, inclusive, by Elvius¹³, Visvanathan¹⁴, Babadzhanyan *et al.*¹⁵, Kinman *et al.*¹⁶, and unpublished observations by the author. This polarisation is characterised by quite large changes which can occur on time scale as short as a few days. To smooth out such changes in a search for systematic effects, annual means of the normalised Stokes parameters (Q_m/I , U_m/I) were formed and used to compute the annual mean polarisation P and the annual mean position angle θ of the optical E-vector. These are shown in Fig. 1; the error bars are the r.m.s. deviations of these means. The distribution of the residuals ($\Delta[Q/I]$, $\Delta[U/I]$) from these means is shown in Fig. 2. Systematic trends (for example, skewness in the distribution of these residuals) may be present, but seem small enough for the means (Q_m/I , U_m/I) to have enough significance for our present purpose. These annual means and their residuals corre-

spond roughly to the slowly varying component B and the rapidly varying component C in the more elaborate analysis by Kinman *et al.*¹⁶. In the top panel of Fig. 1, the monthly means of the optical flux S are given in mJy; they were derived from B -magnitudes given by Kinman *et al.*¹⁶, Barbieri and Abati-Erculiani¹⁷, Lü¹⁸, Babadzhanyan *et al.*¹⁵, Tritton and Selmes¹⁹, and unpublished observations by the author using the relation

$$\log_{10} S = 6.67 - 0.4 B$$

Two substantial outbursts are seen. One began in October 1966 (Kinman *et al.*¹⁶) and ended in 1968, and the second has its maximum in the second half of 1971. The sparse data available from old plates (Angione²⁰) do not contradict the idea that outbursts of this kind are occurring at a rate of about one every five years. It has been suggested by C. Barbieri, G. Romano, S. di Serego and M. Zambon (personal communication) that an actual period of 4.38 yr is present. It does not seem unlikely that these outbursts are associated with the large injections of electrons that give rise to the expansion phenomenon on Sander's¹¹ model. An injection event rate much higher than this would presumably result in the superposition of several expanding radio sources at one time. If the rate was much lower, the expansion phenomenon would be rarer than we observe.

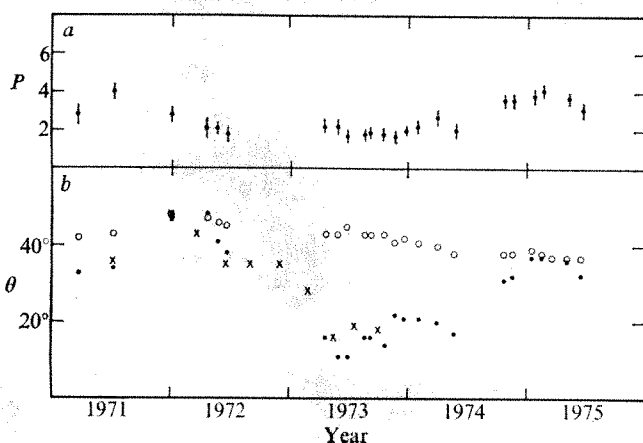
The optical position angle shows some regularity in its behaviour—lying between 80° and 110° when these major outbursts occur. The emission in a highly polarised source of synchrotron radiation must come from an optically thin plasma and will have the E-vector perpendicular to the magnetic field. The field associated with the optical radiation is therefore perpendicular to the expansion direction and (on Sander's model) to the axis of a dipole field. If we speculate that the central source is a massive body with its rotation axis parallel to the axis of the dipole field, the optical radiation could come from an equatorial field on the massive object. In order to account for periodic effects in the optical flux of 3C 345, Kinman *et al.*¹⁶ speculated that the slowly varying component B (which is large when the optical flux is high) arises in the equatorial zone of a rotating body.

Radio polarisation

Radio polarisation data from 1971 to 1975 are shown in Fig. 3. A rotation measure of 18.3 rad m^{-2} (Haves²¹) was applied to the 3.7- and 11.1-cm data of Altschuler and Wardle²², and the 6-cm data of Seielstad and Berge²³. On Sander's model, we would expect the relevant magnetic field to lie predominantly parallel to the expansion direction, and so would expect a position angle for the radio linear polarisation of 15° if the plasma is optically thin, or 105° in the optically thick case. If the field is a permanent structure, the position angle should not show a large secular trend, and this is observed. While the optically thin case does agree better with the observations, it does not seem possible to apply the usual criteria of an expanding cloud model (particularly if beaming effects are present) to decide whether the source is, in fact, optically thin or not. We only note that if it is optically thin for most of this time, then the excursion of the position angle for the 3.7- and 6.0-cm data during 1973 and 1974 (and the simultaneous drop in the percentage polarisation) can be understood in terms of the transient appearance of an optically thick component at these wavelengths.

The present optical and radio data are therefore compatible with models of 3C 345 which involve a relatively permanent structure, and in which sustained relations between the polarisation planes of the radiation and the expansion direction may be expected. Clearly, in any one object, the agreement could be entirely fortuitous, and the

Fig. 3 Radio linear polarisation properties of 3C 345: *a* the percentage polarisation [at 3.7 cm, and *b*, the position angle (corrected for Faraday rotation) at 11.1 cm (○), 6 cm (×), and 3.7 cm (●).



crucial test will be to see if similar relationships can be found in other QSOs. It can also be said that the presence of long term systematic polarisation effects does not give support to QSO models that involve an array of unrelated sources.

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A gating signal for the potassium channel?

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It is suggested that some part of the intramembrane charge movement in striated muscle is associated with the opening and closing of the potassium channel.

THE control of several membrane processes has been associated with small asymmetric displacement currents which may signal intramembrane charge displacement or dipole movement. Schneider and Chandler¹ reported a charge movement in striated muscle which they suggested could be associated with activation of contraction or possibly with activation of potassium conductance². Armstrong and Bezanilla³ and Keynes and Rojas⁴ have investigated the 'gating' currents associated with the activation of sodium conductance in the giant axon of the squid. We report here experiments on the charge movement in striated muscle (sartorius muscle, *Rana temporaria*) which show that it may be separated into two components; one has approximately first order kinetics, and the other has kinetics which seem to associate it with the potassium channel.

Measurement of charge movement

Sartorius muscles were examined in a solution designed to minimise all ionic currents and to prevent contraction. The solution was made hypertonic with sucrose and contained tetraethylammonium ion (TEA) and tetrodotoxin to eliminate sodium current and potassium current⁵. The composition of the solutions was Rb₂SO₄, 5 mM; TEA Cl, 15 mM; (TEA)₂SO₄, 80 mM; CaSO₄, 8 mM; sucrose, 350 mM; TTX, 10⁻⁷ M (ref. 6). Three electrodes inserted close to the pelvic end of a fibre were used to apply exponentially blunted steps of potential ($\tau = 1$ ms, duration 128 ms) to the membrane at the end of the fibre¹. Records of the total current (I) injected into the fibre, the membrane current (i_m) as the potential difference (ΔV) between the two potential measuring electrodes ($i_m = 2\Delta V / 3l^2r_i$, where l is the electrode separation), and the membrane potential (V) at a distance l from the end of the fibre were taken by digitalising the signals and filing them on the disk of a PDP 11 computer. Using this information appropriate programmes calculated the linear membrane constants and displayed nonlinear membrane currents on the computer oscilloscope. Photographic records were also taken of the analogue signals from the fibre to display the complete sequence of prepulses and measuring pulse. Membrane resistance and internal longitudinal resistance (r_i) were calculated from the steady-state values of I , V , and ΔV from a 10-mV control step. The membrane capacity was calculated from the internal

resistance (r_i) and the integral of the transient membrane current (ref. 6)

$$C_{\text{eff}} = \frac{2}{3l^2r_iV} \int_0^\infty \left[\Delta V_i(t) - \frac{\Delta V(\infty)}{V(\infty)} V(t) \right] dt \quad (1)$$

All experiments were done at 2–4 °C.

The detection of charge movement is best done by subtracting the membrane currents required for two identically shaped steps of potential. One of these steps, the test step, is in a range of membrane potential where charges or dipoles are moved by a change of potential: the other, the control step, is ideally in a range where the charges are not moved. For instance one can apply two pulses of different magnitude both starting from the same potential, a potential at which the charge movement is saturated. Alternatively one can apply two pulses of the same size but starting from different potentials. If the steps are not the same size the currents require appropriate scaling before subtraction. The subtraction removes currents in linear membrane elements; linear ionic currents and linear polarisation current in the membrane dielectric due to induced dipoles. Movement of charged molecules or dipole reorientation will in general be nonlinear if a sufficient potential range can be explored. Nevertheless, the subtraction method can only give the difference between the charge moved in test and control pulses, so that if charge moves in the control pulse because the pulses are at insufficiently separated potentials or because there are several species of charges moving the interpretation of nonlinear current may be difficult.

The central sets of records in Figs 1 and 2 are proportional to the non-linear membrane current ($i_m'(t)$) which is defined by

$$i_m'(t) = \frac{2}{3l^2r_i} \left[\Delta V_i(t) - \frac{V_i(\infty)}{V_c(\infty)} \Delta V_c(t) \right] \quad (2)$$

where the subscripts t and c refer to test and control potential steps. In Fig. 1 both control and test steps start at -90 mV: the control step is +10 mV and the positive going test step is variable. This procedure with four control steps and one test step is the same as that used by Adrian and Almers⁸ to study the charge movement they called Charge 1. In Fig. 2 the control pulse is +10 mV from -90 mV: the test pulse is +10 mV from a membrane potential displaced in a positive direction by

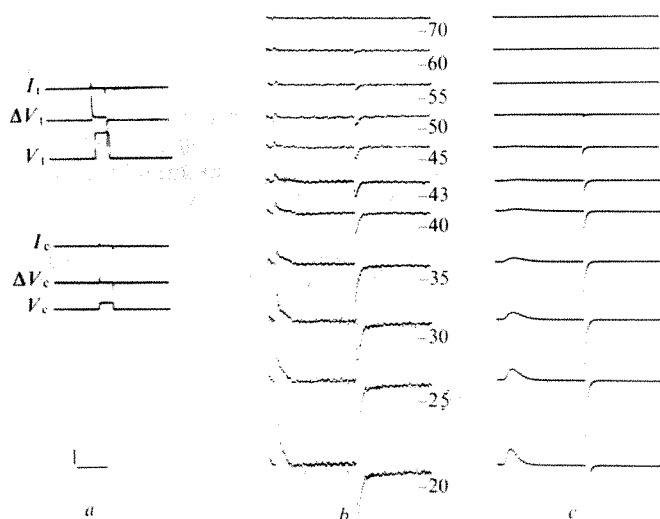


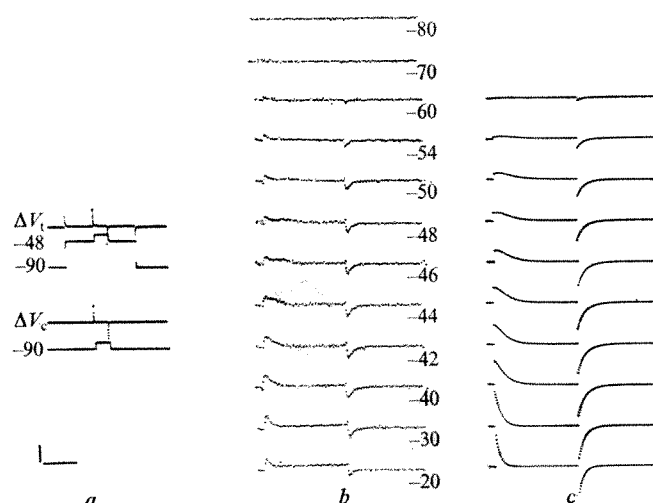
Fig. 1 *a*, Records of I , ΔV and V show a representative sequence of test and control pulses. *b* and *c* were generated by a PDP 11 computer and photographed from an oscilloscope. *b* Shows asymmetrical displacement currents

$$i'_m(t) \times \frac{3l^2 r_i}{2} = \left[(\Delta V_i(t) - \Delta V_c(t)) \frac{V_i(\infty)}{V_c(\infty)} \right]$$

produced by test pulses to an internal potential of -70 , -60 , -55 mV and so on. *c* Shows $d(n^4)/dt$ calculated for steps from -90 mV to -70 , -60 , -55 mV and so on (see text). Calibration lines for the computed traces are 50 ms for both traces; 10 mV for experimental traces and 0.033 ms^{-1} for $d(n^4)/dt$. Fibre 1501: l , 500 μm ; r_i , $21 \pm 0.7 \text{ M}\Omega \text{ cm}^{-1}$; R_m , $11.4 \pm 0.4 \text{ k}\Omega \text{ cm}^{-2}$; C_m , $6.0 \pm 0.1 \text{ }\mu\text{F cm}^{-2}$; diameter, $48 \pm 0.8 \text{ }\mu\text{m}$; temperature, 3.7°C . Fibre constants are means ($\pm$ s.e.m.) of five control steps of 10 mV at beginning of experiment.

a prepulse. In both Figs 1 and 2, the central column of records (*b*) shows the non-linear currents which result during and after the pulse when the potential range from -90 mV to 0 mV is explored. As has been reported previously^{2,8}, integration of the transient non-linear current for the 'on' and the 'off' of the step gives approximately equal charge movements, suggesting a capacitative origin for the current.

Fig. 2 As in Fig. 1, but the asymmetric displacement currents are generated by subtracting the ΔV records for two 10 mV steps starting from different potentials. Calibration lines are 50 ms; 3.3 mV for experimental records and 0.01 ms^{-1} for $d(n^4)/dt$. Fibre 1302: l , 500 μm ; r_i , $13.5 \pm 0.6 \text{ M}\Omega \text{ cm}^{-1}$; R_m , $8.7 \pm 0.3 \text{ k}\Omega \text{ cm}^{-2}$; C_m , $5.6 \pm 0.2 \text{ }\mu\text{F cm}^{-2}$; diameter, $61 \pm 1.5 \text{ }\mu\text{m}$; temperature, 2.5°C .



In both Figs 1 and 2 the nonlinear current is most easily described in terms of two components. For increasing depolarisation during the test step (Fig. 1) or for increasing depolarisation preceding the test step (Fig. 2) one sees first a transient current which is maximal immediately following the 'on' of the step and decays more or less exponentially with a time constant of a few milliseconds. The reverse movement at the 'off' of the step is similarly exponential. At about -50 mV one begins to see a hump of current which occurs first in the middle of the step (that is maximum at about 60 ms from the beginning of the step). At the same potential there is a marked increase in the size of the exponentially decaying current at the 'off'. The hump of current is also clearly visible in the analogue record of ΔV (left hand column of Fig. 2). Over the next 10 mV of depolarisation the hump of current occurs earlier and becomes shorter and higher until eventually both 'on' and 'off' current transients decay more or less exponentially. Figure 1 shows the measured charge movements which take place between -90 mV and a series of more depolarised potentials: Fig. 2 shows the measured charge movements which take place in successive 10 mV intervals of the potential range -80 mV to -10 mV.

In both Figs 1 and 2 at membrane potentials between about -45 mV and -35 mV, the charge movement during the pulse can be described in terms of an exponentially decaying component together with a slower component which rises slowly and then falls. There is, however, no way to separate the components unambiguously and in particular no way of knowing whether, if there are two components, the initial value of the slowly developing movement is finite or zero. Nevertheless, it is evident that the slower charge movement during the voltage step is not well described by a first order process, though the movement after the voltage step might be.

The potassium conductance (g_K) is known to turn on with high order kinetics and off with first order kinetics^{7,9}. It seemed worthwhile, therefore, to see how far the charge movements in Figs 1 and 2 could be predicted by Hodgkin-Huxley kinetics¹⁰. In their description of the potassium conductance changes in the squid axon

$$g_K = \bar{g}_K n^4 \quad (3)$$

$$dn/dt = (1-n)\alpha_n - n\beta_n \quad (4)$$

$\bar{g}_K$ is a limiting conductance and α_n and β_n are rate constants depending only on the membrane potential (V). n^4 can be thought of as the probability that a potassium channel is in the open configuration. Adrian, Chandler and Hodgkin⁷ described the potassium conductance measured in sartorius muscle fibres at $1-3^\circ\text{C}$ with equations (3) and (4) and the rate constants at 3°C

$$\alpha_n = \frac{0.0044 (V+40)}{1 - \exp[-(V+40)/7]} \text{ ms}^{-1} \quad (5)$$

$$\beta_n = 0.0185 \exp[-(V+40)/40] \text{ ms}^{-1} \quad (6)$$

Figure 3 (which is reproduced from ref. 7) shows α_n , β_n , n_∞ (or n_0) and τ_n^{-1} where n_0 and n_∞ are given by

$$n_0 = \frac{\alpha_{n0}}{\alpha_{n0} + \beta_{n0}} \quad (7)$$

$$n_\infty = \frac{\alpha_{n\infty}}{\alpha_{n\infty} + \beta_{n\infty}} \quad (8)$$

and

$$\tau_n = \frac{1}{\alpha_{n\infty} + \beta_{n\infty}} \quad (9)$$

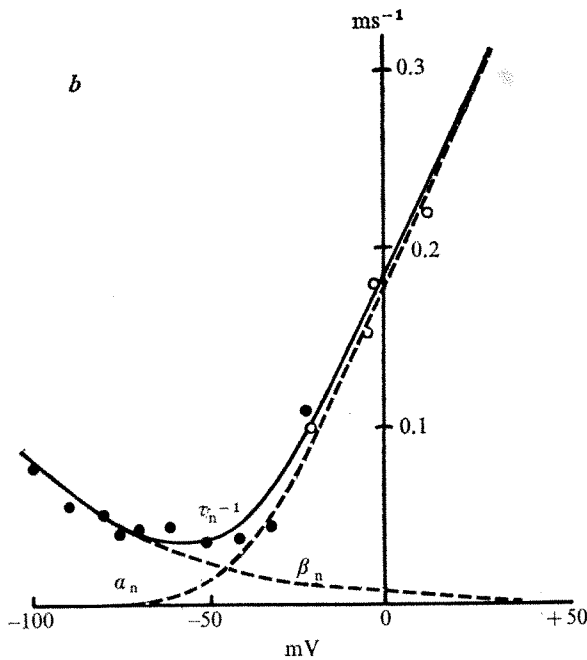
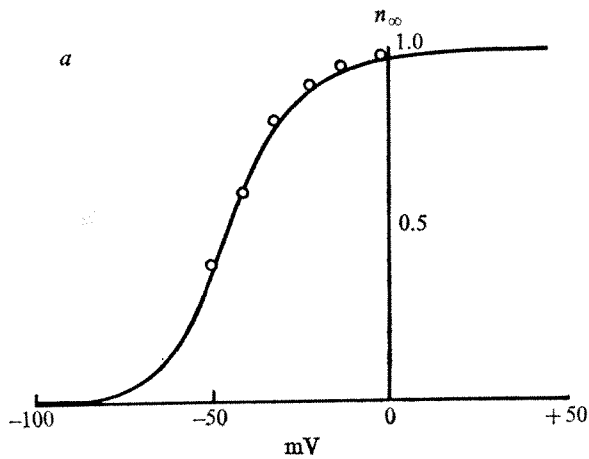


Fig. 3 *a*, Relation between n_{∞} (from steady-state potassium conductance) and membrane potential. Open circles represent experimental points: the line is calculated from

$$n_{\infty} = \frac{\alpha_n}{\alpha_n + \beta_n}$$

b, Relation between rate constants (τ_n^{-1}) obtained from records of potassium currents produced by depolarisation (○) and repolarisations (●) to different potentials: the line is calculated from

$$\tau_n^{-1} = \alpha_n + \beta_n$$

The dotted lines are calculated by equations (5) and (6). (Figure reproduced from ref. 7.)

For a step change of potential from V_0 to V_{∞} the solution to equation (4) is

$$n = n_{\infty} - (n_{\infty} - n_0) \exp(-t/\tau_n) \quad (10)$$

Hodgkin and Huxley¹⁰ suggested that movement of four independent particles onto a potassium channel precedes its opening. The movement of each n particle was described by equation (4). On this basis one would expect to detect an exponentially decaying charge movement (i_n) whenever the potential was changed abruptly which would be given by

$$i_n \propto (dn/dt) = \tau_n^{-1} (n_{\infty} - n_0) \exp(-t/\tau_n) \quad (11)$$

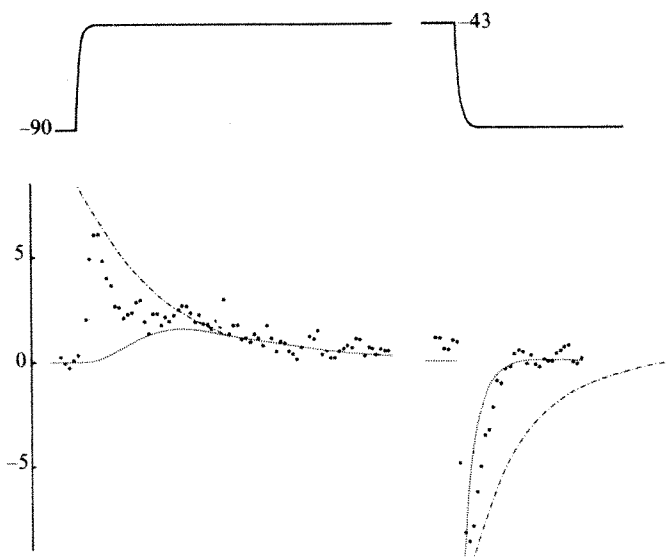
If the actual opening of a potassium channel involved the redistribution of other charges in the membrane field, or the entry of additional charges into the membrane, then in addition to the charge movement reflecting the preliminary and rate-limiting process there would be a charge movement associated with the opening process. This would resemble

$$\begin{aligned} \frac{d}{dt}(n^4) = & \frac{4(n_{\infty} - n_0)}{\tau_n} \left[n_{\infty}^3 \exp\left(\frac{-t}{\tau_n}\right) \right. \\ & - 3n_{\infty}^2 (n_{\infty} - n_0) \exp\left(\frac{-2t}{\tau_n}\right) + \\ & + 3n_{\infty} (n_{\infty} - n_0)^2 \exp\left(\frac{-3t}{\tau_n}\right) \\ & \left. - (n_{\infty} - n_0)^3 \exp\left(\frac{-4t}{\tau_n}\right) \right] \quad (12) \end{aligned}$$

Different kinetic models could be made to generate different combinations of exponential terms. Equation (12) by itself could not describe the total charge movement since it implies that the n process does not involve charge movement and therefore could not be potential dependent.

Figure 4 plots $i_m'(t)$ from Fig. 1 at a test potential of -43 mV and compares with it dn/dt and $d(n^4)/dt$ calculated by equations (5) to (12) for a potential step from -90 mV to -43 mV at a temperature of 4°C (Q_{10} for rate constants was assumed to be 2.5). The two calculated curves have been arbitrarily scaled. It is clear that the charge during the test step could be described by an arbitrary combination of dn/dt and $d(n^4)/dt$. But it is equally clear that this would not be a unique description. It would, however, be difficult to describe the charge movement in terms of either function alone.

Fig. 4 The points are values of $[3I^2 r_1 i_m'(t)]/2$ derived from a control step of $+10$ mV and a test step of $+47$ mV both from -90 mV. The dash-dot line is dn/dt , the dotted line $d(n^4)/dt$ both calculated from equations (5) to (12) at 4°C . They are arbitrarily scaled so that the curves coincide in the latter part of their time course during the voltage step. Note that although the voltage step was blunted with a time constant of 1 ms, the calculated curves assume a sudden step. The points which are taken from the experiment in Fig. 1 are at 1-ms intervals on the horizontal axis: the vertical scale is in mV.



The right-hand columns of Figs 1 and 2 give $d(n^4)/dt$ calculated for the voltage steps of the experimental records. They show the extent to which later parts of the charge movements resemble the differential of n raised to a power greater than the first. In Fig. 1, $d(n^4)/dt = 0$ at the beginning of the step since n_0 at -90 mV is effectively zero. In Fig. 2 at the beginning of the 10 mV test step n_0 is not equal to zero and for $t = 0$

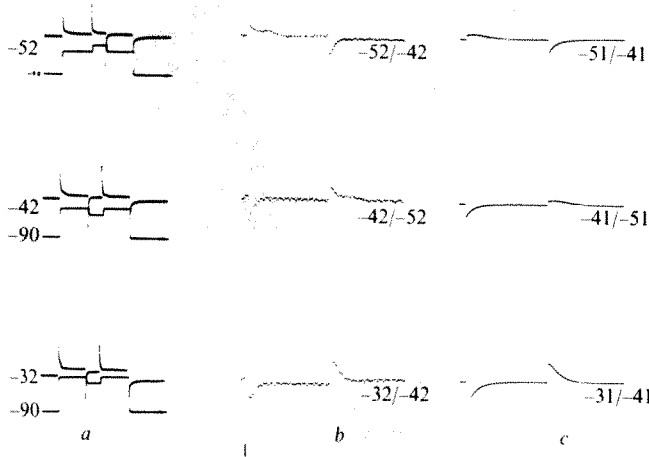
$$\frac{d}{dt}(n^4) = \frac{4n^3_0(n_\infty - n_0)}{\tau_n}$$

Figure 5 extends the similarity between part of the detected charge movement and $d(n^4)/dt$. In Fig. 5a and b charge is moved between the same potentials but in opposite directions: both charge movement and $d(n^4)/dt$ are approximately exponential when the potential change is negative, $d(n^4)/dt$ and part of the charge movement are humped when the potential change is positive. Figure 5c shows the charge movement when the potential changes from -32 to -42 mV: Fig. 5a shows the charge movement at the same potential but when the potential changes from -52 to -42 mV. Though the charge movement takes place at the same potential its time course depends on the direction of movement. The same kind of difference is seen in $d(n^4)/dt$.

Separation of charge movement into components

The question arises whether all or only part of the initial and approximately exponential charge movement seen in the experimental records should be associated with the more complex later charge movement. It would be difficult from the records to separate the charge movement into components. But in striated muscle it is known that the potassium conductance inactivates with time. This inactivation resembles, but is much slower than, the inactivation of the sodium conductance. Adrian, Chandler and Hodgkin¹⁷ have shown that the potassium conductance is about 70% inactivated when the membrane potential is held at -40 mV. If the membrane potential is held at -40 mV, but stepped to -90 mV for a 'control' +10 mV pulse and to other membrane potentials for 'test' 10 mV pulses, then the detected charge movement seems to consist of an early exponential component only. The voltage distribution of this moved charge (Q against V curve) does not resemble n_∞ being less steeply voltage dependent and with a transition potential at about -15 to -20 mV. The shape of this curve

Fig. 5 As in Fig. 2, but showing that configuration of displacement current depends on its direction. Calibration lines are 50 ms; 3.3 mV for experimental computer traces and 0.01 ms⁻¹ for $d(n^4)/dt$. Fibre 1303; l , 500 μ m; r_i , 11.8 ± 1.3 M Ω cm⁻¹; R_m , 14.3 ± 0.6 K Ω cm⁻²; C_m , 11.9 ± 0.2 μ F cm⁻²; diameter, 66 ± 3.2 μ m; temperature 2.6 °C.



resembles that postulated by Adrian, Chandler and Rakowski¹¹ to account for repriming of contraction after long depolarisation.

If the membrane potential is held at -20 mV both contraction and potassium conductance are inactivated and if charge movement is examined over the same potential range (-90 mV to 0 mV), both early exponential and late slow charge movements are absent. This effect of holding potential charge movement was first reported by Chandler, Rakowski and Schneider¹². It seems possible therefore that part, at least, of the early and exponential charge movement may be related to contraction and that the later slow charge movement as well as an undetermined part of the early more or less exponential charge movement may be related to the potassium channel.

In what follows we shall refer to this latter current as the opening signal or current, making the plausible but unproved assumption that it is a signal from the potassium channel. The implication is that external TEA blocks the potassium channel by combining with an external site on the channel^{5,13} but leaves unaltered the opening and closing of an internally located gate. The opening of this gate is associated with a movement of charge in or perhaps into the membrane: its closing with an equal and opposite movement of charge.

How many charges move when one channel opens? To estimate this quantity we need to know the conductance of a single potassium channel. We shall assume a value between 10^{-12} and 10^{-11} S (ref. 14). If the total opening charge is 10 nC μ F⁻¹ and the membrane capacity is 5 μ F cm⁻² the charge is 50 nC cm⁻² of fibre surface. When this amount of charge has moved, in the absence of TEA the potassium conductance would be 20 mS cm⁻² (ref. 7). If the channel conductance is 4 pS (ref. 15) each opening event is associated with the movement of 60 charges. This figure is no more reliable than the rather uncertain quantities on which it is based, but it seems to exclude the possibility that the opening current is due to the entry of an internal TEA ion or other internal ion into the blocked channel when the gate opens¹⁶. Equally, though it might be consistent with models which involve the association of several multiply charged molecules in the opening process, the magnitude of the opening signal is unexpectedly large¹⁷.

In previous studies the charge movement in normally polarised muscle has been called charge 1 (refs 2, 8, 17). Chandler, Rakowski and Schneider^{2,12} gave careful consideration to an association between charge 1 and potassium activation but felt that there was insufficient evidence for a firm conclusion. Nonetheless, though comment has been made on the complexity of its kinetics¹⁸, charge 1 has been tentatively considered to be one species of charge and more likely to be associated with contraction activation than potassium channel activation¹⁷. Our results suggest that charge 1 may be two distinct charge species. Movement of one of these charges is an approximately first order process and this charge remains a candidate for being a regulator of contraction activation. The second charge could be associated with the potassium channel. The kinetics of this second charge movement favour an association with the opening of the potassium channel. But the size and complex time course argue against any simple link of the kind suggested by Hodgkin and Huxley¹⁰. Moreover, it is still possible that the detected charge movement is related to some high order process other than potassium gating.

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DNA complementary to parathyroid mRNA directs synthesis of pre-proparathyroid hormone in a linked transcription–translation system

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DNA complementary in sequence to the messenger RNA for pre-proparathyroid hormone was synthesised using reverse transcriptase. In a linked transcription–translation system using RNA polymerase and cell-free extract from wheat germ, the DNA directed the synthesis of a protein identified as pre-proparathyroid hormone by N-terminal sequencing and by electrophoretic and immunologic criteria.

PARATHYROID hormone (PTH) is a small polypeptide of 84 amino acids that is of critical importance in the regulation of calcium levels in the extracellular fluid of higher vertebrates (see ref. 1 for review). In the parathyroid gland, PTH is first synthesised as a precursor of 115 amino acids, pre-proparathyroid hormone (Pre-ProPTH) (ref. 2). During or shortly after synthesis *in vivo*, Pre-ProPTH undergoes two consecutive proteolytic cleavages, resulting in the formation of PTH through an intermediate precursor, parathyroid hormone, a polypeptide of 90 amino acids³. Both proteolytic cleavages occur at the amino-terminal end of the precursors. The primary structures of PTH and the two biosynthetic precursors have been determined^{4–6}.

Previously we have prepared and characterised the messenger RNA for Pre-ProPTH (refs 4, 5, 7). Using reverse transcriptase, we have now synthesised DNA complementary in sequence (cDNA) to this message. Recently, several investigators^{8–10} have developed conditions for using reverse transcriptase to synthesise cDNA molecules as large as their mRNA templates. We have used similar conditions and report here that the cDNA is large enough to direct the synthesis of Pre-ProPTH in a linked transcription–translation cell-free system. The cDNA was transcribed using wheat germ RNA polymerase, and the newly synthesised RNA was then used to direct protein synthesis in a cell-free extract. Control experiments showed that this synthesis was not due to contamination of the cDNA by the initial mRNA. The Pre-ProPTH synthesised was characterised by molecular sizing on polyacrylamide gels, specific reactivity with antiserum to PTH, and amino-acid sequencing at the N terminus.

DNA-directed Pre-ProPTH synthesis

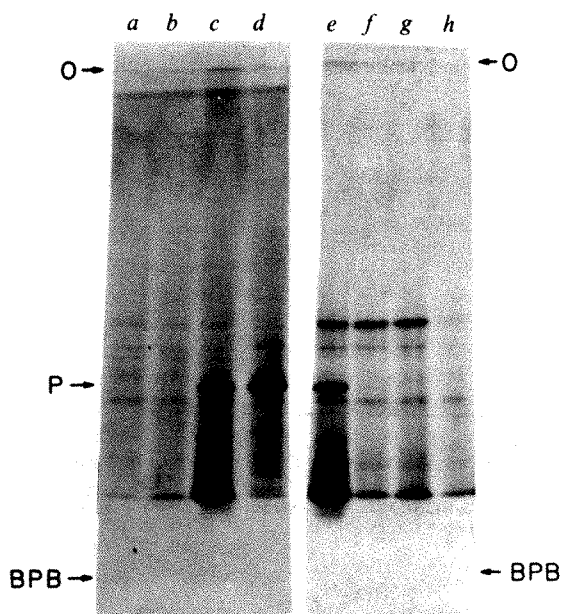
Complementary DNA was synthesised from partially purified Pre-ProPTH mRNA using conditions similar to

those described by Efstratiadis *et al.*⁸ (see legend to Fig. 1). Electrophoresis in a 5% polyacrylamide–98% formamide gel showed that the cDNA is heterogeneous in size, with a major species containing almost 800 nucleotides. Its characterisation will be described more fully elsewhere. The Pre-ProPTH mRNA was not characterised in detail here, but earlier studies indicated that it is approximately the same size as the cDNA (refs 4, 6).

We tested the ability of the cDNA prepared from Pre-ProPTH mRNA to direct the synthesis of Pre-ProPTH in the linked transcription–translation system. After treatment with alkali to hydrolyse the RNA, the cDNA stimulated the synthesis of a protein that co-migrated with Pre-ProPTH on a SDS–urea, 15–20% polyacrylamide gradient gel (Fig. 1c, d). The synthesis of this protein was dependent on the addition of wheat germ RNA polymerase (Fig. 1b). Moreover, all of the proteins synthesised in the absence of RNA polymerase co-migrated with those that were synthesised by the wheat germ extract in the absence of any added nucleic acid (Fig. 1a, b).

The incorporation of ³H-UTP into trichloroacetic-acid-precipitable material was dependent upon the addition of RNA polymerase to the cDNA (Table 1). It was reasonable to conclude that this newly-synthesised RNA was translated into Pre-ProPTH, but other possible alternatives had to be examined. For example, the polymerase might have stimulated the synthesis of non-functional RNA that protected from degradation some of the original mRNA present as a contaminant in the cDNA preparation. Therefore, experiment 2 in Fig. 1 was performed in the presence of carrier transfer RNA (1 mg ml⁻¹ during the transcription), and the results make this explanation unlikely. The synthesis of Pre-ProPTH required the presence of RNA polymerase (Fig. 1e, g), even though carrier tRNA was included in each incubation. Alternatively, the polymerase might have removed contaminating mRNA from a cDNA–mRNA hybrid, allowing the mRNA to bind ribosomes and undergo translation. These possibilities and most other explanations that postulate contamination of the cDNA preparation with mRNA were eliminated by treating the cDNA preparation with DNase. Complementary DNA treated with pancreatic DNase during the transcription did not stimulate protein synthesis in the linked transcription–translation system (Fig. 1e, f). The possibility that the effect of DNase was not due to digestion of cDNA, but rather due to the action of contaminating RNase was excluded. In the conditions of the incubations, mRNA added separately to the reaction with DNase retained 84% of its original activity (Table 1).

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contained 2 A_{260} units ml^{-1} Pre-ProPTH mRNA, four deoxyribonucleotide triphosphates, 1 mM each, and ^3H -dCPT adjusted to 0.4 Ci mmol^{-1} . Avian myeloblastosis virus reverse transcriptase was kindly supplied by J. W. Beard. After 3 h at 43 °C, the reaction mixture was adjusted to 0.1 N in NaOH and incubated for another 16 h at 37 °C. After neutralisation of the NaOH with an equal amount of HCl and adjustment to 0.1 M in Tris-HCl (pH 9.0), the nucleic acid was extracted once with an equal volume of phenol-chloroform-isoamyl alcohol and passed over a 5 ml Sephadex G1-50 column in H_2O . Fractions eluting in the void volume of the column were pooled, adjusted to 0.1 M NaCl and precipitated with 2 volumes of ethanol. In experiment 2, but not in experiment 1, a mixture of purified wheat germ tRNA provided by T. Fraser, was added at 50 $\mu\text{g ml}^{-1}$ as a carrier before the ethanol precipitation. The conditions used for the linked transcription-translation reactions were similar to those described previously^{11, 12} except that the transcription reaction was carried out for 1 h instead of 15 min. Each reaction mixture contained approximately 0.1 μg DNA, estimated from the specific activity of ^3H -CTP. In experiment 2 (channels (e)-(h)), assuming 100% recovery, the tRNA added as carrier was present at a concentration of 1 mg ml^{-1} during transcription and 0.2 mg ml^{-1} during translation. Addition of larger amounts of wheat germ tRNA to the reaction mixture inhibited translation. Different preparations of cDNA were used in each of the two experiments. ^{35}S -methionine was used as the radioactive amino acid. After the translation reaction was completed, the proteins in 5 μl of each 50 μl extract were analysed by electrophoresis on 15–20% polyacrylamide, SDS-urea gels²⁴ followed by fluorography²⁵. Gels were exposed to Kodak SB-5 film for 3 d at -70 °C. Experiment 1: channel a, no DNA; b, no wheat germ RNA polymerase added; c, cDNA and RNA polymerase added; d, 0.04 μg mRNA added directly to 50 μl of the translation reaction mixture. Experiment 2: channel e, cDNA, RNA polymerase and tRNA added; f, same as (e) plus pancreatic DNase 0.1 mg ml^{-1} added during transcription; g, same as (e) without RNA polymerase added; h, same as (e) without cDNA or tRNA. O, origin; P, PreProPTH; BPB, bromophenol blue dye front.

Fig. 1 Polyacrylamide gel analysis of two experiments demonstrating Pre-ProPTH synthesis in a linked transcription-translation system. Calf parathyroid glands were frozen in liquid nitrogen within 30 min of killing. Subsequently, they were ground to a powder in a mortar chilled with dry ice. While still frozen, 10 volumes of 0.05 M Na acetate (pH 5.0), 0.01 M EDTA, 0.5% SDS, and heparin (100 $\mu\text{g ml}^{-1}$) were added along with an equal volume of phenol:chloroform:isoamyl alcohol (1:1:0.4) and the mixture was allowed to thaw to room temperature. All extractions were carried out at room temperature. Three extractions with the phenol:chloroform:isoamyl alcohol mixture were performed, followed by three extractions with chloroform:isoamyl alcohol (24:1), and finally by precipitation with two volumes of ethanol overnight at -20 °C after adjustment to 0.2 M with NaCl. The nucleic acid was dissolved in 0.5 M NaCl, 0.01 M Tris-HCl (pH 7.5), 0.5% SDS, then passed over a column containing 1.0 ml of oligo-dT-cellulose (T-3, Collaborative Research) for every 50 A_{260} units of nucleic acid. (1 A_{260} defined as the amount of nucleic acid that has an absorbance of 1 at 260 nm, when dissolved in 1 ml of water and measured through a 1-cm light path.) The column was eluted with 0.01 M Tris-HCl (pH 7.5), 0.5% SDS. The eluant was adjusted to 0.5 M NaCl, bound to and eluted from the column of oligo-dT a second time, precipitated in ethanol, and then centrifuged through a linear gradient of 5–20% sucrose in buffered 50% formamide as described²³. Fractions collected from the gradient were assayed for mRNA coding for Pre-ProPTH using the wheat germ cell free system⁸, and fractions containing maximum activity were pooled. The predominant, but not exclusive protein made from this preparation of mRNA was Pre-ProPTH (channel d). cDNA was synthesised using the method of Efstratiadis *et al.*⁸. The reactions

Characterisation of protein synthesised in the linked system

We took advantage of the detailed characterisation of Pre-ProPTH (refs 4–6) to investigate the structure of the protein synthesised from cDNA. Immunoprecipitation of the products from the linked transcription-translation reaction with guinea pig antiserum to PTH and rabbit anti-guinea pig serum produced a polypeptide that co-migrated on sodium dodecyl sulphate (SDS)-urea polyacrylamide gels with authentic, mRNA-directed Pre-ProPTH (Fig. 2). Moreover, no ^{35}S -methionine-labelled peptide comigrated with Pre-ProPTH in immunoprecipitates prepared from cell-free reactions lacking either cDNA or RNA polymerase. The cDNA-directed product was not precipitated when treated with non-immune serum and rabbit anti-guinea pig serum or when bovine PTH (35 $\mu\text{g ml}^{-1}$) was included before the addition of anti-PTH antiserum and rabbit anti-guinea pig serum. The specificity of the immunological studies was strengthened further by control experiments in which anti-PTH antiserum and rabbit anti-guinea pig serum did not precipitate any of the numerous ^{35}S -methionine polypeptides synthesised in the linked system in response to SV40 DNA, form I (ref. 12). Thus, our cDNA-directed product contains antigenic determinants that are present in PTH.

Methionine is present at positions 1, 2, 7, 11 and 14 in the amino-terminal region of Pre-ProPTH (ref. 4). We therefore used Edman degradation to determine the location

of the methionines in the structure of the cDNA-directed product that was labelled with ^{35}S -methionine (Fig. 3). ^{35}S -methionine was released during the degradations at cycles 1, 2, 7, 11 and 14, a pattern identical to that found in a similar sequence analysis of mRNA-directed Pre-ProPTH. Thus, sequence studies, immunological data, and electrophoretic analysis indicate that the structure of the cDNA-directed product is very similar, if not identical, to the structure of Pre-ProPTH. We conclude that the cDNA that we have synthesised contains a faithful representation of the structural information for Pre-ProPTH and can express this information in the linked, transcription-translation system.

Discussion

The linked transcription-translation system provides an important method for assessing the fidelity and completeness of the cDNA products of the reverse transcription of mRNA. Furthermore, the method is independent of hybridisation and sizing tests. Figure 4 summarises the reactions required to synthesise Pre-ProPTH in the linked transcription-translation system. The synthesis of cDNA is presumed to begin at or near the 3'-terminus of the mRNA with an oligo-dT primer and clearly extends to the nucleotides encoding the N-terminal methionine of Pre-ProPTH. Therefore, the cDNA must include the complement of any untranslated region at the 3' end of the mRNA (region C in Fig. 4). It must also span the region coding

Table 1 cDNA-directed RNA and protein synthesis in the linked transcription-translation system

Experimental conditions	Trichloroacetic-acid insoluble radioactivity	
	RNA- ³ H c.p.m.	Protein- ³⁵ S c.p.m. × 10 ⁻³
Experiment 1		
<i>a</i> No nucleic acid	2,320	226
<i>b</i> No RNA polymerase	2,060	266
<i>c</i> cDNA-directed product	10,820	1,010
<i>d</i> mRNA-directed product		538
Experiment 2		
<i>e</i> cDNA-directed product		599
<i>f</i> DNase (0.1 mg ml ⁻¹) included along with cDNA		240
<i>g</i> No RNA polymerase		240
<i>h</i> No nucleic acid added		202
<i>i</i> mRNA (0.2 µg) added at time of transcription		1,447
<i>j</i> As (<i>i</i>) plus DNase (0.1 mg ml ⁻¹)		1,231

Experimental conditions detailed in Fig. 1 legend. RNA synthesis was measured using ³H-UTP (0.1 Ci mmol⁻¹). 0.5 µl of the 10 µl transcription reaction was counted before the addition of translation reagents. The values for radioactivity refer to the total (10 µl) in the reaction mixture. The RNA radioactivity in reactions (*b*) and (*c*) was derived by subtracting the known amount of ³H-cDNA (8,200 c.p.m.) added to each reaction from the total radioactivity in the reactions (10,260 and 19,020 respectively). ³⁵S-methionine (460 Ci mmol⁻¹) was used in the translation reactions. 1 µl of the 50 µl translation reaction was measured. The radioactivity in the Table refers to the total 50 µl. The reactions (*a*)–(*h*) correspond to reactions (*a*)–(*h*) in Fig. 1.

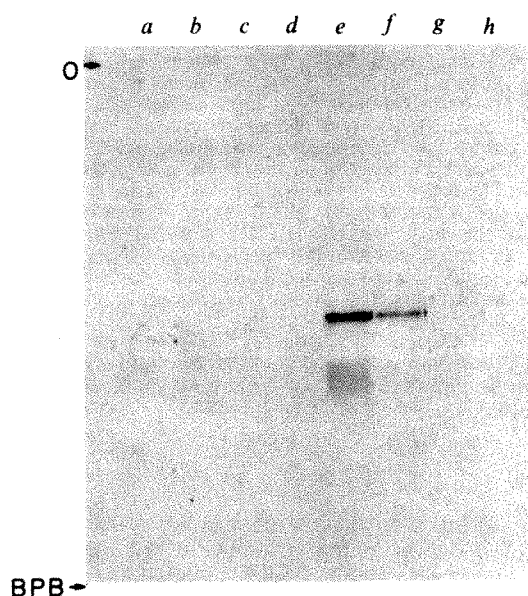


Fig. 2 Indirect immunoprecipitation of Pre-ProPTH. Approximately 100,000 c.p.m. of ³⁵S-methionine-labelled protein from each reaction were added to 200 µl 0.05 M veronal buffer (pH 8.5), 1% foetal bovine serum. Guinea pig anti-PTH serum (GP1) (ref. 26), diluted to a final concentration of 1:1000, was added to each tube except (*d*). After 24 h at 4 °C, 50 µl of rabbit anti-serum to guinea pig globulin (Arnel) were added to each tube, and the tubes were incubated at 4 °C for an additional 24 h. The resulting precipitates were collected by centrifugation at 1200g for 30 min, washed twice with 2 ml of 0.15 M NaCl, 0.02 M Tris-HCl (pH 7.5) and dried. The proteins were analysed by electrophoresis on an SDS-15–20% polyacrylamide slab gel²⁷. The gel was fluorographed²⁸ (channel *f* for 1 d, all the rest for 10 d) at -70 °C using Kodak SB-5 film. Immunoprecipitates of the following reactions are displayed: channel *a*, no cDNA or RNA polymerase added; *b*, cDNA added without RNA polymerase; *c*, cDNA and RNA polymerase added and non-radioactive PTH (35 µg ml⁻¹ during immune precipitation) added along with anti-PTH serum; *d*, cDNA and RNA polymerase added and non-immune serum substituted for anti-PTH serum at the same dilution; *e*, cDNA and RNA polymerase added; *f*, 0.08 µg mRNA added directly to wheat-germ translation reaction; *g*, 0.1 µg SV40 DNA (form I), and RNA polymerase added; *h*, same as *g* but non-radioactive PTH (35 µg ml⁻¹ during immune precipitation) added along with anti-PTH antiserum. O, origin; BPB, bromophenol blue front.

for Pre-ProPTH (region B), and reach at least the initiator codon at the 5' end of the mRNA.

If region A contains sequences necessary for message

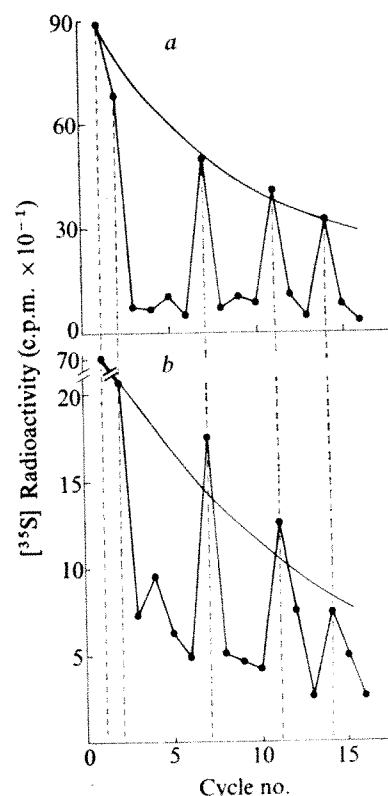


Fig. 3 Sequence analyses of *a*, ³⁵S-methionine-labelled Pre-ProPTH synthesised in response to parathyroid mRNA in the wheat-germ cell-free system and *b*, the major ³⁵S-methionine-labelled protein synthesised in the linked transcription-translation system in response to parathyroid cDNA. The Pre-ProPTH (*a*) was isolated by electrophoresis on an SDS-urea polyacrylamide gel²⁴ and the cDNA-directed protein by indirect immunoprecipitation with an antiserum to PTH. The radiolabelled proteins were subjected to automated Edman degradation (Beckman 890 sequencer) in the presence of 2 mg of apomyoglobin added as carrier. Aliquots of one-third of the total radioactivity released at each cycle of degradation were assayed directly for radioactivity as the butyl chloride extract. The identity of the radioactive amino acids was confirmed by chromatography of the remainder of each fraction on thin layer plates as described previously³. The continuous curved line represents the theoretical recoveries of methionine on each cycle based on the efficiency of degradation³.

activity in the linked transcription-translation system, then our cDNA must extend into region A. Unfortunately, there is no firm information about the absolute requirement for

this region in our experiments. Shine and Dalgarno¹³ have shown that prokaryotic mRNA's contain sequences that are complementary to a sequence on 16S ribosomal RNA. Binding studies¹⁴ using mRNA from R17 bacteriophage and *Escherichia coli* ribosomes suggest that this sequence is functionally important. Ptashne *et al.*¹⁵ have suggested, however, that ribosomal-binding sequences on mRNA may not be absolutely required for mRNA activity. They have demonstrated that the bacteriophage λ repressor mRNA transcribed from the promoter p_{RM} does not require a 5'-terminal extension beyond the initiator AUG-codon for translation, albeit inefficient translation, *in vivo*. Furthermore, the functional importance of ribosomal-binding sequences¹⁶ or other sequences 5'-proximal to the initiator nucleotides has not been determined for eukaryotic messages. Thus we cannot be certain about the completeness of the polynucleotide sequence of our cDNA in region A.

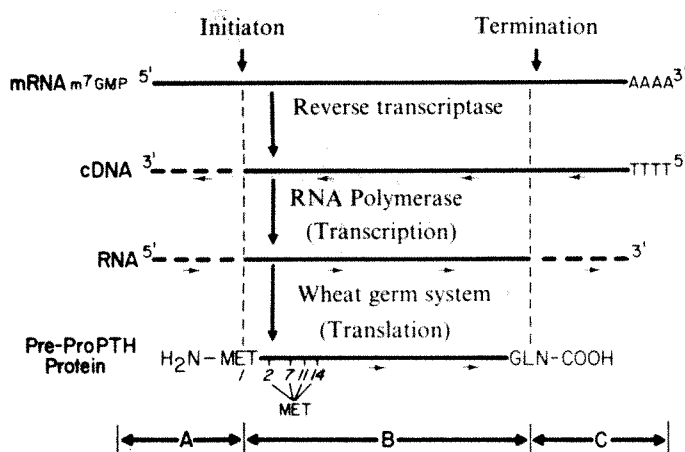


Fig. 4 Diagram of cDNA synthesis and synthesis of Pre-ProPTH in the linked *in vitro* transcription-translation system. Horizontal solid lines indicate sequences necessarily synthesised in the functioning linked system. Dotted lines indicate sequences that may have been synthesised. The N-terminal methionines of the Pre-ProPTH synthesised in the linked system are indicated. Horizontal arrows under cDNA, RNA, and Protein denote the direction of synthesis of these molecules.

Our demonstration that RNA synthesised in the linked transcription-translation system is accurately translated into Pre-ProPTH raises interesting questions regarding the role of the 7-methyl guanosine terminal in the activity of parathyroid mRNA. Kemper¹⁷ has reported that treatment of Pre-ProPTH mRNA sequentially with periodate and aniline inactivates the message almost completely. Control studies suggested that periodate and aniline were effective at least partly because they removed 5'-terminal 7-methyl guanosine¹⁷. These studies of Kemper and more recent data from Rose and Lodish¹⁸ demonstrate that periodate and aniline treatment can inactivate messages by producing alterations in the mRNA other than removal of 7-methyl guanosine. Therefore, whether or not the Pre-ProPTH mRNA absolutely requires a 7-methyl guanosine terminus in order to be translated is unresolved. In our studies either 'uncapped' RNA is translated or a 7-methyl guanosine cap is added to the RNA by the linked transcription-translation system. Analysis of the newly-synthesised RNA should resolve this issue.

We are as yet uncertain as to whether or not our functional cDNA is single or double-stranded. Using conditions similar to those that we have followed, Efstratiadis *et al.*⁸ found that only 9.2% of their globin cDNA was double stranded, as defined by resistance to the S1 nuclease, which is specific for single-stranded nucleic acid. This fraction became even smaller (4.4%) when actinomycin D

was added during reverse transcription. We did not include actinomycin in our reaction mixture during synthesis of cDNA and cannot be sure how much of our DNA is double-stranded. Conceivably, even a small amount of double-stranded DNA could be responsible for all meaningful transcription. Further studies using actinomycin D and techniques for separating single and double-stranded DNA are underway.

The methodology described here should be particularly useful for investigators attempting to introduce functional DNA into bacteria²⁰⁻²². At each stage of synthesis and modification of the DNA, the DNA can be monitored to assure that the nucleic acid retains sufficient information to code for appropriate proteins. After the vector has been successfully introduced into bacteria, restriction fragments of the recombinant DNA can be similarly assayed. If the particular protein of interest is not synthesised by the bacteria, the linked system should allow the detection of potentially functional sequences not properly transcribed by the bacteria. An additional strength of this methodology is that the cDNA employed in the studies need not be pure. Crude cDNA mixtures, which can later be purified by cloning, can be shown to contain the structural information for the proteins of interest.

The parathyroid cDNA is a suitable starting material for nucleotide sequence analysis²³. These data should be especially interesting in regions A and C of Fig. 4. Further, the synthesis of double-stranded parathyroid DNA should provide a substrate for detailed restriction endonuclease analysis. The ligation of specific DNA restriction fragments could generate DNA molecules with characteristic deletions and insertions. The behaviour of these modified DNA's in the linked transcription-translation system should allow a detailed analysis of the function of specific untranslated nucleotide sequences. Similar experiments can be carried out in the coding region B to create modified versions of PTH whose activities could be assayed in biological systems.

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Mechanism of acid protease catalysis based on the crystal structure of penicillopepsin

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A proposed mechanism for the catalytic hydrolysis of peptide bonds by acid proteases is similar in many respects to the Zn-carbonyl mechanism previously derived for carboxypeptidase A. In the acid proteases the electrophilic component is the proton shared by Asp-32 and Asp-215; Tyr-75 donates its proton to the amide nitrogen of the scissile bond and an OH⁻ ion from a water molecule bound between the carboxyl group of Asp-32 and the substrate attacks the carbonyl carbon atom.

ACID proteases catalyse the hydrolysis of peptide bonds at acidic pH values. The solutions of high resolution structures of three of these enzymes isolated from diverse microbial sources have been reported^{1,2}. These crystal structures will be of fundamental importance in elucidating the catalytic mechanism of acid proteases. The electron density map of penicillopepsin¹ is exceptionally well defined and the region of the active site is unambiguous in its interpretation. In addition, this is the only acid protease for which both the primary structure and crystal structure have been determined. We are therefore in a position to propose a mechanism based on structural information and previously documented chemical data.

Our primary concern in the present discussion is the bond-breaking step of polypeptide substrate hydrolysis by acid proteases. Further studies with substrate analogues, inhibitors and virtual substrates will add to our present interpretation of the catalytic mechanism and should provide the details of the enzyme-substrate interactions of elongated substrate peptide chains.

The history of studies of the catalytic mechanism has been most recently reviewed by Fruton³. In that article a number of proposals for future research were also made. We present here additional data which could help in the interpretation of those experiments. Moreover, neither a covalent amino-enzyme nor a covalent acyl-enzyme intermediate is required by the mechanism described here. The existence of these covalent intermediates was essential to many of the previous hypotheses but the intermediates have not been detected.

Crystal data

Penicillopepsin crystallises in the monoclinic system (space group, C2, with unit cell dimensions $a = 97.38(8)$, $b = 46.62(3)$, $c = 65.39(6)$ Å and $\beta = 115.4(3)^\circ$) from a 9 mg ml⁻¹ solution of the enzyme and 2.5 M Li₂SO₄, 0.1 M sodium acetate at pH 4.4. The low-resolution crystal structure at 6 Å showed that the molecule was decidedly asymmetric with dimensions

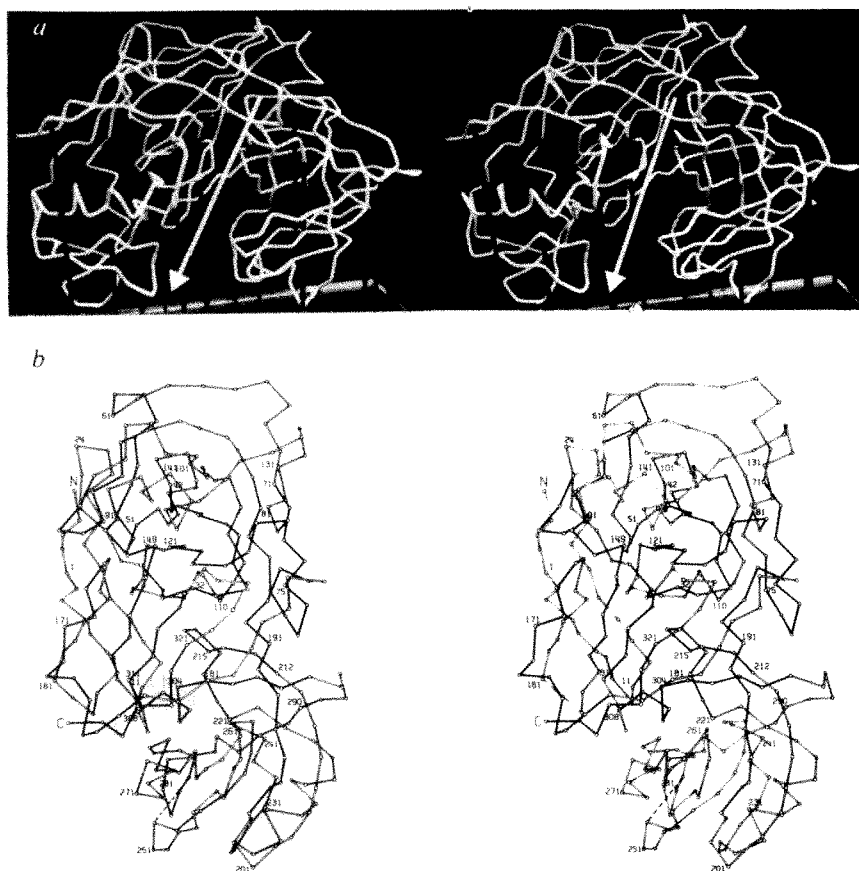


Fig. 1 *a*, Stereoscopic representation of the penicillopepsin α -carbon model. The arrow is shown in the extended substrate binding cleft which divides the molecule into two approximately equal halves (the N-terminal domain is to the left, the C-terminal domain to the right). Asp-32 (in the N-terminal domain) and Asp-215 (in the C-terminal domain) have their α -carbon positions in a dark colour. *b*, Stereoscopic drawing (from the program ORTEP of Dr Carroll Johnson) of the polypeptide chain. The view is from the top of that shown in (*a*). Every tenth α -carbon atom has been assigned a number corresponding to the sequence given in ref. 1. The active site region is that region shown with black connectivity; Asp-32 and Asp-215 are labelled.

$65 \times 49 \times 39 \text{ \AA}$ (ref. 4). Intensity data to $2.8 \text{ \AA}$ resolution (6,700 unique reflections) were obtained from a single crystal of native penicillopepsin and each of the eight heavy-atom derivatives used for determining phases by the method of multiple isomorphous replacement (MIR). The data collection protocol is discussed in more detail in refs 5 and 6 and the statistics regarding the phase determination are given in ref. 1. The overall mean figure of merit of the 6,127 statistically significant reflections was 0.90 (for zero error in phase angle this index has a value of 1.0). Observed native structure factor amplitudes and the 'best' phases⁷ were used to compute the electron density function of the penicillopepsin molecule. This map was interpreted using the partial sequence data⁸ for 16 fragments of penicillopepsin. In this way, it was possible to correlate the majority of the 322 residues in the polypeptide chain with the sequence data. Eight amino acid residues, however, were assigned from electron density distribution alone¹.

Structure and function

Figure 1 shows two different representations of the α -carbon model of penicillopepsin. A deep cleft separates the two lobes of the molecule and the active site is located within this cleft. A more detailed description of the polypeptide chain conformation is given in ref. 1. The positions of Asp-32 and Asp-215 are marked in the figures as an aid to orientation. The extensive hydrophobic core (in the shape of a croissant) appears as a relatively 'empty' region surrounded by the 18-strand mixed β -sheet, the major structural determinant of penicillopepsin. This hydrophobic core is highly ordered in the electron density map and the aromatic and aliphatic side chains are absolutely unambiguous in their assignment.

1,2-Epoxy-3-(*p*-nitrophenoxy) propane binding to penicillopepsin

The reagent 1,2-epoxy-3-(*p*-nitrophenoxy) propane (EPNP) is a specific inhibitor of acid proteases and has been used to identify the carboxylate group of Asp-32 at the active site⁹. Direct proof that Asp-32 and Asp-215 are present in the active site of penicillopepsin comes from the following experiment. A single crystal of penicillopepsin was soaked for 15 d in a saturated solution of EPNP in standard buffer at pH 4.4. The $2.8\text{-\AA}$ diffraction intensity data were collected from this crystal and a difference electron density map was computed from the amplitudes ($|F_{\text{EPNP}}| - |F_p|$) and the MIR phases (α_p). This map had significant density in only two regions. One region corresponds to what we interpret as a non-covalently-bound EPNP molecule in a solvent cavity between two protein molecules in the vicinity of residues 27–29 and 116–117. The second and more interesting region involves residues in the active site. This portion of the difference map is shown in Fig. 2. The positive values of difference density, indicated by solid lines, show that two molecules of EPNP have reacted covalently with penicillopepsin, one with the carboxyl group of Asp-32, the other with the carboxyl group of Asp-215. In addition, Fig. 2

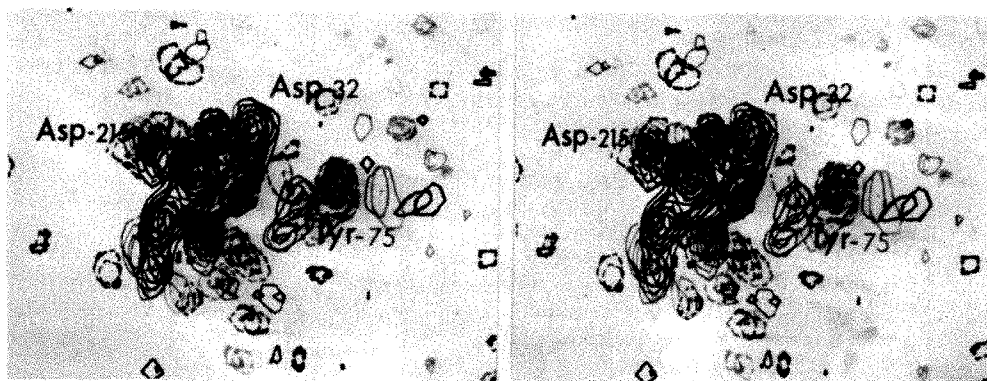
shows a concomitant pronounced conformational change of Tyr-75 in which the side chain phenoxyl group moves from a position where it was hydrogen-bonded to Trp-39 (an invariant residue in penicillopepsin and pepsin) to one in which it lies coplanar with the *p*-nitrophenoxy moiety of the EPNP molecule bound to Asp-32.

Room temperature studies of Chen and Tang¹⁰ on the inhibition of porcine pepsin with EPNP resulted in two peptides which had covalently bound EPNP; one with the sequence Asp(EPNP)-Thr-Gly-Ser-Ser-Asn (residues 32–37) and the other with sequence Ile-Val-Asp(EPNP) (residues 213–215). A similar experiment at 4°C by Hartsuck and Tang⁹ resulted in only one EPNP reactive peptide which corresponded to the Asp-32 sequence. Chen and Tang¹⁰, perhaps in view of the low temperature results, chose to assign the EPNP reactive site only to Asp-32. Their observations and our present knowledge of the sequence are in good agreement with the fact that both Asp-32 and Asp-215 have reacted with EPNP in penicillopepsin crystals at room temperature.

Although it is possible to accommodate two EPNP molecules simultaneously in this active site region of penicillopepsin, it could also be that the binding of one EPNP molecule to either of Asp-32 or Asp-215 would preclude the binding of the second EPNP molecule and that we are actually observing statistical disorder in the crystals. This is unlikely, as the two peaks of electron density (Fig. 2) are of approximately equal size, and the interplanar separation of the *p*-nitrophenoxy moieties is of the order of $3.5 \text{ \AA}$, a reasonable value for aromatic rings. The residues of penicillopepsin that are close to the EPNP molecule bound to Asp-215 are those residues that are likely to be responsible for binding natural peptide substrates in the S_1 site of penicillopepsin. These residues are Leu-222, Thr-218, Ile-301 and a segment of the hairpin loop from Asn-290 to Ile-297 (see Fig. 1).

The conformational change observed for Tyr-75 is of particular interest because of the analogy of this movement with that observed in substrate binding with carboxypeptidase A_a (refs 11, 12). Tyr-75 is located on a hairpin loop (β -bend) which involves residues 70–83 (see Fig. 1). In the penicillopepsin electron density map, the density corresponding to this 'flap' is very well defined because this region represents a close intermolecular contact between two penicillopepsin molecules across a twofold symmetry axis in the crystals. There is a third strand which anchors the flap into a three stranded antiparallel β -sheet. These strands do not form part of the major 18-stranded β -sheet, but they are made up of stretches of β -chain conformation from residues, 70–77; 78–83; 105–108. Although the conformational changes induced on EPNP binding involve only Tyr-75 (rotation around the C_α - C_β bond), it is probable that this whole 'flap' region undergoes a major conformational change to accommodate the binding of longer substrate molecules. This would be the reason for the CD spectral changes observed when binding certain activators to penicillopepsin¹³.

Fig. 2 Stereoscopic photograph of the difference electron density map of the covalent inhibitor EPNP (see text). Positive values of difference density are represented as solid contours, negative values with dashed contours. The two EPNP molecules bind to the carboxyl groups of Asp-32 and Asp-215 and the *p*-nitrophenoxy moieties are coplanar and point towards the bottom of the photograph. Since this is a difference map the electron density of the carboxyl groups is not seen, but lies approximately where the side chains are designated (see Fig. 3a). The conformational movement of Tyr-75 is seen to the right of and below the positive density of the EPNP molecule bonded to Asp-32.



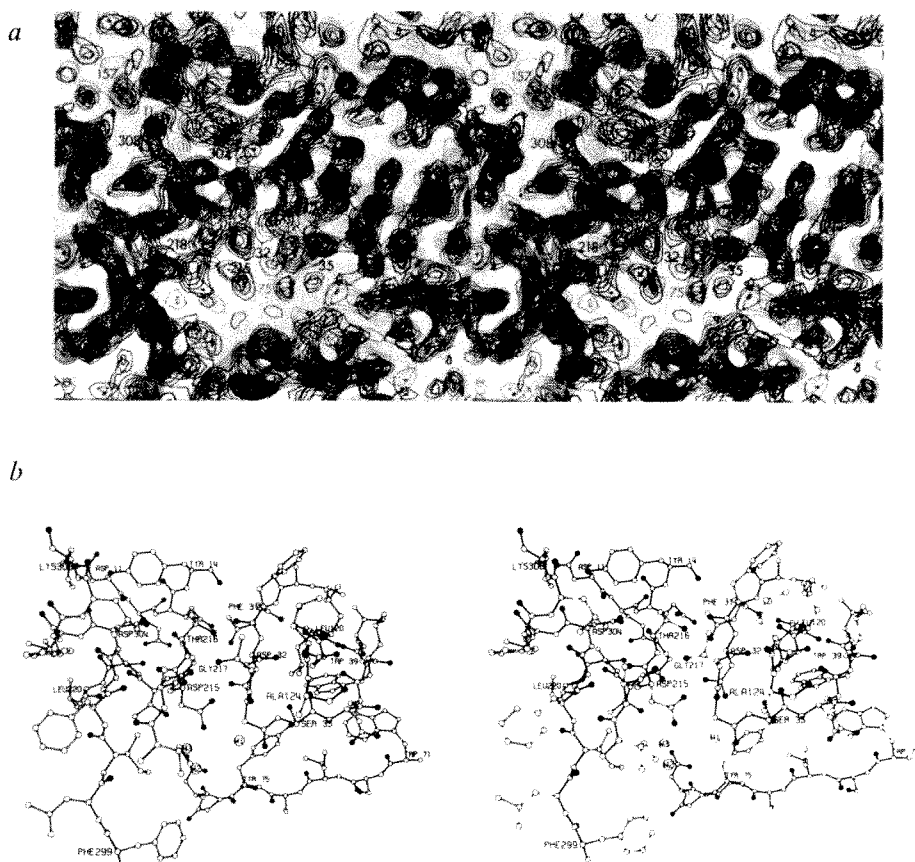


Fig. 3 *a*, Electron density map of penicillopepsin in the region of the active site. Side chains of the amino acids discussed in text have been labelled with the numbering given in ref 1. *b*, Schematic interpretation of the electron density map shown in 3*a* showing the disposition and hydrogen-bond interactions between the several groups in the region of the active site (portions with black connectivity in Fig. 1*b*). The bound water molecules are labelled W(1) W(2) and W(3).

A final point regarding the EPNP studies concerns the difference of the nature of the covalent penicillopepsin-EPNP complexes with Asp-32 and Asp-215. The mechanism of ring opening of 1,2-epoxides in acid solution favours products in which the nucleophile attacks the 2 position of the propane moiety¹⁴. The electron density associated with the EPNP molecule bonded to the carboxyl group of Asp-215 is consistent with this mechanism as there is difference density on either side of the carboxyl group. But, the difference electron density of the EPNP molecule which has reacted with Asp-32 clearly terminates at that group on the enzyme and the complex is Asp-32 (EPNP) with the carboxyl group bonded to the 1 position of the propane moiety. This fact indicates that the carboxyl group of Asp-32 is inaccessible for the formation of covalent intermediates with peptide substrates without major conformational changes in the enzyme's tertiary structure. A negative peak on the difference map in the region between the two carboxyl groups of Asp-32 and Asp-215 shows that the hydrogen-bonded complex of these two groups in the native enzyme structure has been disrupted by their reaction with EPNP.

Disposition of groups in the active site of penicillopepsin

Before considering the catalytic mechanism of acid proteases, we feel it is important to emphasise further that the two carboxyl groups located in the active site are relatively inaccessible to substrate molecules, and that most of the region is inflexible to gross conformational changes. A carboxylate anion (Asp-32) and a protonated carboxyl group (Asp-215) have been implicated in the catalytic mechanism of acid proteases (ref. 3 and references cited therein). The sections of the electron density map which contain these groups are shown in Fig. 3*a*. Figure 3*b* is a schematic representation of the corresponding part of the molecule. It can be seen that the carboxyl group of Asp-32 is in an unusually tight hydrogen-bonded environment reminiscent of the hydrogen-bonded environment of Asp-102 of the serine

proteases¹⁵. The contacts that are made to the carboxyl group of Asp-32 include (1) the carboxyl group of Asp-215, (2) the O⁻ of Ser-35, (3) the main chain N-H of Gly-217 and (4) a bound water molecule W(1). Although not seen in Fig. 3, the main chain from Asp-32 to Gly-34 traverses directly above (towards the viewer) the carboxyl group of Asp-32 and makes several close contacts with this moiety.

The carboxyl group of Asp-215 is more exposed to solvent than is the carboxyl group of Asp-32. The side chain of Thr-218 is located to the left and below (away from the viewer in Fig. 3*a*) the COOH group of Asp-215 but no direct hydrogen bonding exists between these two side chains. Two water molecules, W(2) and W(3), are within hydrogen-bonding distance of Asp-215. The side chain Ile-213 is seen above and to the left of Asp-215 and in conjunction with the side chains of Phe-189 and Tyr-299 probably forms the S₁' hydrophobic binding site in penicillopepsin¹⁶. These residues correspond to Ile-213, Tyr-189 and Leu-299 respectively, in pepsin¹⁷.

There are other residues in the neighbourhood of the two active site aspartic acid groups. The peptide bond from Thr-216 to Gly-217 lies between the two carboxyl groups of Asp-32 and Asp-304. The carboxyl group of Asp-304 (Fig. 3), deeply buried in the hydrophobic core of the molecule, makes a hydrogen-bonded contact with the main chain C=O of Thr-216. To the left of Asp-304 there is an ion-pair, formed by Lys-308 and Asp-11, which has neighbouring residues Tyr-14 and His-157 to its right and left respectively. Tyr-14 is also buried in the hydrophobic core and the hydroxyl group lies within 3.2 Å of Asp-304 and the ion-pair Lys-308 to Asp-11. All of the above mentioned residues with the exception of His-157 are conserved in the porcine pepsin sequence^{1,17}.

In addition to these side chain interactions it is important to consider the polypeptide chain conformation in the vicinity of the active site aspartic acid residues. Both residues are in regions of chain that show a high degree of not only primary structural homology but also tertiary structural homology¹. The chain from Phe-31 to Ser-35 adopts a similar conformation

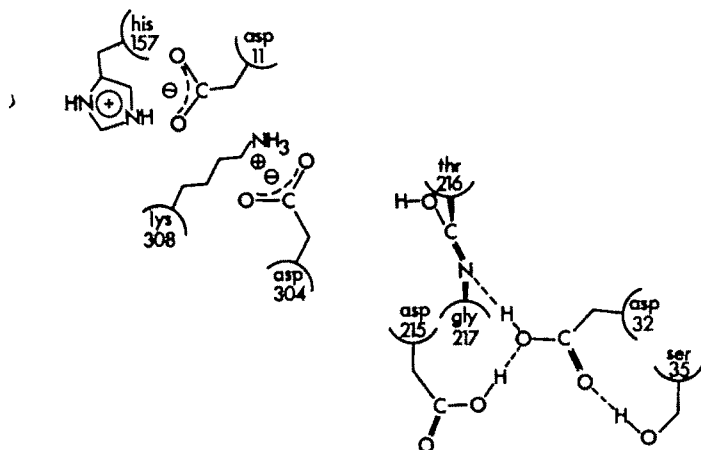


Fig. 4 Proposed tautomeric form of the peptide bond between Thr-216 and Gly-217 which could result from conformational changes induced on the binding of extended substrate peptides to penicillopepsin. The net effect is to enhance the electrophilic character of the proton between Asp-215 and Asp-32.

to the segment of chain from Ala-214 to Thr-218. (There is in fact much more extensive tertiary structural homology between the two lobes of penicillopepsin, but it is not pertinent to the discussion here and will be considered in a subsequent publication). Asp-32 is in strand c (strand nomenclature for penicillopepsin and the corresponding residue numbers within strands are given in ref. 1), with nearest neighbours strand h (running parallel to c) and strand m (anti-parallel to c) which contains Asp-215. In addition to strand c, strand m (containing Asp-215) has a parallel strand p which is its other nearest neighbour strand. These main chain conformations and the fact that the strands are in an extensive mixed β sheet which has a pronounced hydrophobic side, lead one to the inference that the active site region is conformationally rigid and that movement of the two lobes relative to one another would not make the active site much more accessible to substrate. In support of this, it has been observed that pepsin and penicillopepsin retain partial catalytic activity in 8 M urea (T. Hofmann, unpublished).

The immediate surroundings of the two active site carboxy groups are consistent with the observations that Asp-32 has a low pK_a and Asp-215 has a high pK_a , and the latter would be predominantly protonated at pH values from 2 to 4. As this proton is shared between these two carboxyl groups, however, there would be little driving force for it to act in the role of facilitated proton transfer to the substrate carbonyl oxygen atom in the manner suggested by Knowles¹⁸. The presence of the carboxyl group of Asp-304 adjacent to the peptide bond between Thr-216 and Gly-217 offers a charge relay system which is the major factor giving this shared proton an electrophilic character. Enhanced electrophilicity of this proton may be achieved by the mechanism depicted schematically in Fig. 4. Asp-11 is at the extremity of a hairpin loop (strands a and b, ref. 1) in the central portion of the large mixed β -sheet region that would be involved with the binding of an extended substrate molecule. A relatively minor conformational change of this loop would disrupt the Asp-11 to Lys-308 ion pair, and at least in penicillopepsin, Asp-11 could form an alternative ion pair with His-157 which is on the surface of the enzyme and is protonated at pH values < 5 . This induced conformational change would leave an almost completely buried positive charge of Lys-308 in the hydrophobic interior. This energetically unfavourable situation could be relieved by a subsequent conformational change in which the carboxyl-group of Asp-304 protonates the main chain carbonyl oxygen atom of Thr-216 and rotates by $\sim 120^\circ$ about the $C_\alpha-C_\beta$ bond to form an internally buried ion-pair with Lys-308. The protonation of the peptide bond between Thr-216 and Gly-217 would then produce

the tautomer as shown in the diagrammatic sketch in Fig. 4. Support for this proposal comes also from chemical studies of Kitson and Knowles¹⁹ and Huang and Tang²⁰ in which it was observed that chemical modification of arginine residues of pepsin resulted in a loss of catalytic activity. Chemical modification of Lys residues of penicillopepsin results in a similar loss of activity (T. Hofmann, unpublished). As the only basic residue that is in common between porcine pepsin and penicillopepsin¹ is at position 308 we suggest that Arg-308 is the modified residue in porcine pepsin.

These conformational changes involving Asp-11, Lys-308 and Asp-304 represent an extreme case in which a very strong acid, the protonated peptide bond between Thr-216 and Gly-217, is formed. We do not suggest that this extreme conformational change is ever reached, however. A partial contribution towards the tautomer shown in Fig. 4 would also result in enhanced electrophilic character of the proton which is bound between the two carboxyl groups Asp-32 and Asp-215, rendering this proton more labile than one would expect in its shared state as seen in the penicillopepsin structure. The degree of electrophilic character of the proton would then be a function of the relative contributions of these two tautomeric forms of the Thr-216 to Gly-217 peptide bond.

Our structural studies have shown that penicillopepsin has an extended substrate binding groove¹. A protein conformational change is known to occur when certain tripeptide activators (Leu-Gly-Leu) bind in a region near Trp-39, Trp-71 and Tyr-75 (ref. 13). Transpeptidation products isolated from reaction mixtures in which the activators are present indicate that this binding region (secondary A site as defined by Takahashi and Hofmann¹⁸) is the N-terminal region of bound substrate, that is, P_1-P_4 . In addition, Fruton has shown that increased substrate chain length in the N-terminal direction from the scissile bond enhances k_{cat} with little difference in substrate binding affinity³. These effects may now be explained by the increased electrophilic character of the proton between the two carboxyl groups of Asp-32 and Asp-215.

Towards a catalytic mechanism for acid proteases

The acyl-enzyme mechanism for serine proteases is well established¹⁸ and the postulated tetrahedral intermediate in that pathway has subsequently been observed in crystalline trypsin-trypsin inhibitor complexes^{21,22}. Catalytic mechanisms for the thiol proteases²³ and metalloproteases¹³ have also been advanced and the proponents of these mechanisms have drawn heavily on the results of crystal structural studies. In the most recent review article on acid protease mechanisms³, Fruton states at the outset "that speculations about the catalytic mechanism of pepsin have been limited by lack of information about the three-dimensional structure of its active site . . .". The electron density map of penicillopepsin at 2.8-Å resolution¹ provides the missing details of the active site geometry and the following proposed mechanism is based on the structural results and on chemical and kinetic studies done with synthetic substrates of acid proteases.

The description of the active site geometry of penicillopepsin in the previous section, by no means exhaustive, was intended to convey two major points: (1) that the β -carboxyl group of Asp-32 is too buried for this group to act as an attacking nucleophile directly, and (2) that the sum of the interactions of the groups in the active site and the conformations of the main polypeptide chains prohibit large conformational changes in this region. A conformational change involving the extensive anti-parallel β sheet which contains Tyr-75, as discussed above, is possible however, and probably accounts for the conformational changes observed on binding certain peptide activators. It is also unlikely that the carboxyl group of Asp-215 acts as an attacking nucleophile even though it is partially exposed to solvent, because it is the carboxyl group with the higher apparent pK_a of 4.7 (ref. 18). How then is one to accommodate peptide bond catalysis by acid proteases?

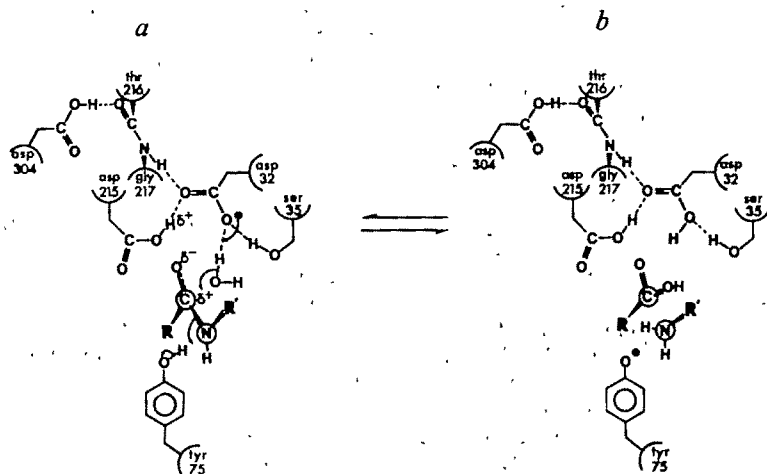


Fig. 5 Proposed general base catalytic mechanism of the acid proteases. *a*, Schematic drawing of the productive binding mode for substrate RCONHR' in the active site of penicillopepsin. The conformational change of the 'flap' region brings Tyr-75 to a position from which it can protonate the amide nitrogen of the scissile bond. Asp-32 attacks the carbon atom of the polarised $C^{\delta+} \cdots O^{\delta-} \cdots H^{\delta+}$ system through the bound water molecule W(1). Transpeptidation through acyl or aminic transfer is a function of the relative affinity of the enzyme for the acyl or amino fragment shown in *b*.

The scheme that we propose is shown in Fig. 5 which contains the essential details of the active site (shown more extensively in Fig. 3). It also includes the scissile peptide bond of a hypothetical substrate RCONHR'. The structure of the binding region, as observed in the Kendrew model of penicillopepsin, is compatible with that orientation of an X-Phe-Phe-Y type substrate (the Phe-Phe bond being cleaved). Side chain interactions of substrate with enzyme have been omitted from Fig. 5 to enhance clarity, but are essentially as described in the previous sections.

The formation of the Michaelis complex is accompanied by a conformational change of the flap region (residues 74–80) which has to open outwards to admit the substrate into the extended substrate binding cleft. We have pointed out an approximation to this change in the EPNP binding experiment, where it was limited to the movement of Tyr-75 by the crystal packing of molecules of penicillopepsin. Substrate binding also displaces water molecules W(2) and W(3), whereas W(1) can remain in its original position. It now lies between the substrate (as postulated) and the carboxyl group of Asp-32. With the substrate bound, the flap can return and in so doing, the phenolic OH of Tyr-75 comes close to the amide of the susceptible peptide bond. The productive binding mode is now complete as depicted in Fig. 5*a*.

It is useful to point out the analogy of this binding mode to the substrate orientation found for glycytyrosine in the carboxypeptidase A study done by Lipscomb and co-workers^{11,12}. In our case, Tyr-75 is in a position to protonate the amide nitrogen atom of the peptide bond (Fig. 5*a*). This function is carried out by Tyr-248 in carboxypeptidase A after a similar conformational change of the phenolic group upon substrate binding. The electrophilic component of the carboxypeptidase mechanism is ascribed to the Lewis acid, Zn^{2+} in the Zn-carbonyl mechanism. Polarisation of the carbonyl bond in the case of acid proteases is accomplished by the proton shared between the two active site carboxyl groups. We propose that the electrophilicity of this proton is a function of the nature of the substrate, and could be enhanced by specific enzyme-substrate interactions as described in the previous section.

Finally, the Zn-carbonyl mechanism for carboxypeptidase A postulates either a direct nucleophilic attack of Glu-270 on the polarised carbonyl carbon of the peptide bond or a general base attack through a bound water molecule. The analogous residue to Glu-270 of carboxypeptidase A is the carboxylate of Asp-32 of the acid proteases. Carboxypeptidase A has a pH optimum close to 7.5 where the Glu-270 side chain would have a carboxylate anion. The special requirement of acid proteases in having an ionised carboxylate at very low pH values are met by the most unusual environment of the Asp-32 side chain. We propose that attack of the polarised carbonyl carbon atom of the scissile peptide bond occurs through the activated water molecule W(1) rather than by direct nucleophilic attack by the

$\beta\text{-COO}^-$ of Asp-32 (Fig. 5*a*). Thus we prefer this general base mechanism of hydrolysis without the involvement of either an anhydride or an amino-enzyme intermediate.

A major stumbling block in the various other proposed mechanisms of acid proteases, as reviewed by Fruton³, has been the inability of workers to trap either of the covalent intermediates (acyl-enzyme or amino-enzyme). Our proposal obviates this problem and postulates a ternary complex of enzyme, acyl-fragment and amino-fragment after cleavage of the susceptible peptide bond. The order of release of products is determined by the relative affinity of the acyl or amino moiety to their respective binding sites²⁴.

Transpeptidation is a phenomenon peculiar to the acid proteases and it has only been observed to proceed to any significant degree at pH values greater than 4.0. Our mechanism is also compatible with the transpeptidation phenomenon. The products resulting from hydrolysis of the peptide bond are most likely the carboxylate ($R\text{-COO}^-$) and the protonated amino group (NH_3^+-R'). Transfer of the proton from the carboxyl group to the amino group would occur readily and the positively charged amino group could be stabilised by the phenoxide ion of Tyr-75 (Fig. 5*b*). As an example, consider the work of Newmark and Knowles²⁴ on pepsin, in which they studied the transpeptidation products from the peptide $^{14}\text{C}\text{-Leu-Tyr-}^3\text{H}\text{-Leu}$. Approximately 80% of the resulting Leu-Leu is $^{14}\text{C}\text{-Leu-}^{14}\text{C}\text{-Leu}$; the remainder is $^3\text{H}\text{-Leu-}^3\text{H}\text{-Leu}$ with none of the mixed radiolabelled product. This tripeptide binds initially such that the N-terminal Leu-Tyr bond is cleaved. Exchange of Tyr-Leu with a second Leu-Tyr-Leu is more facile than the exchange of the bound N-terminal $^{14}\text{C}\text{-Leu}$ product from the first hydrolysis. Transpeptidation would occur through the microscopic reversibility of the mechanism shown in Fig. 5, yielding the Leu-Leu-Tyr-Leu which is subsequently cleaved by a second hydrolytic step to give $^{14}\text{C}\text{-Leu-}^{14}\text{C}\text{-Leu}$. Fruton³ has pointed out that at pH values at which transpeptidation occurs (>4.0) the equilibrium constant for the reaction: $\text{RCOO}^- + \text{H}_3\text{N}^+ - \text{R}' \rightleftharpoons \text{RCONHR}' + \text{H}_2\text{O}$ is 1 and reversal of the hydrolytic step is equally probable. But, at pH values close to the optimum pH of pepsin ($\text{pH} \sim 2$) one would expect the carboxyl of the acyl fragment to be protonated, thus precluding transpeptidation at low pH values.

Conclusions

The mechanism proposed here is consistent with the disposition of amino-acid groups at the active site of penicillopepsin and with various characteristics of acid protease hydrolysis. The electrophilic component of hydrolysis is the shared proton between Asp-32 and Asp-215 (ref. 18). The electrophilicity of this proton is a function of specific enzyme-substrate interactions and its variation is seen in the variation of k_{cat} with closely related substrates. We have not used hypotheses that require covalent acyl or amino intermediates because the

existence of these species has not been detected or conclusively proven. Whether or not transpeptidation proceeds through acyl or amino transfer (indeed both pathways have been observed in the same reaction mixture) is determined by the affinity of the amino or acyl moiety bound to the enzyme following the first hydrolytic step. Transpeptidation proceeds only at pH values where one has charged groups involved with the formation of the new peptide bond. Protonation of the carboxylate at low pH values precludes transpeptidation through this mechanism and favours hydrolysis.

Finally, another convincing argument in favour of this mechanism is the very close similarity to that one proposed by Lipscomb for carboxypeptidase A. It seems that these two enzymes have converged on a mechanism for the hydrolysis of peptide bonds which differs from that of the serine protease mechanism. The specific requirement of catalytic activity in strongly acid media precludes the use of a Lewis acid such as Zn^{2+} . The acid proteases have instead developed a bond-polarising system with a bound proton and a charge-relay system which influences the electrophilicity of this proton. Tyr-75 clearly is very similar to Tyr-248 of carboxypeptidase both in its function of transferring a proton to the amide nitrogen atom and the conformational change it undergoes on substrate binding. Both the metalloproteases^{12,25} and the acid proteases use an ionised carboxylate group to catalyse the conversion of the substrate carbonyl carbon to a transient tetrahedral intermediate which results in the cleaved peptide bond. The proposal here that acid proteases catalyse this step through general base attack through a bound water molecule ($W(1)$) lends support to this step being present as well in the carboxypeptidase A mechanism. It has been pointed out that the carboxypeptidase A mechanism resembles that of pepsin in several respects²⁶ and it is therefore gratifying to find that the structural features of penicillopepsin are consistent with such closely corresponding mechanisms of catalysis.

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Note added in Proof: Completion of the building of the entire model of penicillopepsin has required the following revisions to the sequence reported in ref 1. (a) The molecule is made up of 323 residues. (b) The following residues are now interpreted as: 299 is Phe, 302 is Phe and 249–264 are tentatively assigned as: -Asx-Cys-Ser-Ser-Ser-Val-Pro-Asx-Phe-Ser-Val-Ser-Ile-Ser-Gly-Tyr-.

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letters to nature

Intensity transitions in Cyg XR-1 observed at high energies from OSO 8

THE high energy X-ray source, Cygnus XR-1, exhibits a variable intensity between states which differ in flux at lower energies (typically 3 to 6 keV) by a factor of three (refs 1–5). Although the transitions between these 'high' and 'low' flux states occur at irregular intervals, the transition itself usually takes no more than one day in either direction. We report here an observation of such a transition at energies above 20 keV which shows that the spectrum of Cyg XR-1 exhibits the pivoting effect during intensity transitions expected from two-temperature accretion disk models of the X-ray emitting region^{6,7}.

Cyg XR-1 was observed with the high energy X-ray spectrometer on board the OSO-8 satellite from 11 to 19 November, 1975, and from 27 October to 15 November, 1976 (excluding the period from 1 November to 7 November, 1976). The CsI(Na) scintillation spectrometer is sensitive to radiation in the 20 keV to 3 MeV range. The detector is actively shielded and collimated to a 5-degree circular FWHM and offset by an angle of 5 degrees from the negative spin axis of the satellite. Further details of the instrument and its satellite environment are given by Dennis *et al.*⁸ Once every rotation period of the satellite (~10 s), any source on the detector's scanning circle will transit through its field of view. The data were analysed in the manner outlined by Dolan *et al.*⁹.

The raw counting rates during the 1975 observations in the interval 23 to 153 keV are shown in Fig. 1. Data are grouped in intervals of 0.55995 d, or a phase interval of one-tenth the period of the HDE 226868 binary system¹⁰. A decrease in counting rate of ~40% was observed to occur on 16 November, 1975, with the source remaining at a significantly lower counting rate after the transition than before it. Comparison with 3–6-keV proportional counter data taken by Holt *et al.*¹ and Serlemitsos (quoted in ref. 11) reveals a simultaneous rise in the lower energy counting rate. Boldt¹² distinguishes between 'transitions' and 'transient events'. In transient events, the 3–6-keV intensity fluctuates by a large amount from its previous value but returns to its original level within a few binary periods; in transitions, the flux remains at its new level for many binary periods. We cannot distinguish between these two possibilities with our observations, and shall refer to all large variations lasting more than a few days as transitions. It is apparent that we have observed for the second time at higher energies¹³ a transition between intensity states in Cyg XR-1.

Our observations for 1976 are shown in Fig. 2. The 23–153-keV intensity was higher than that observed in 1975, indicating that a reverse transition must have occurred between the two observations, in agreement with the report of such a transition in February 1976, by Holt *et al.*¹ The decrease observed by us to occur on 14 November 1976, probably does not correspond to a change in intensity state at low energies;

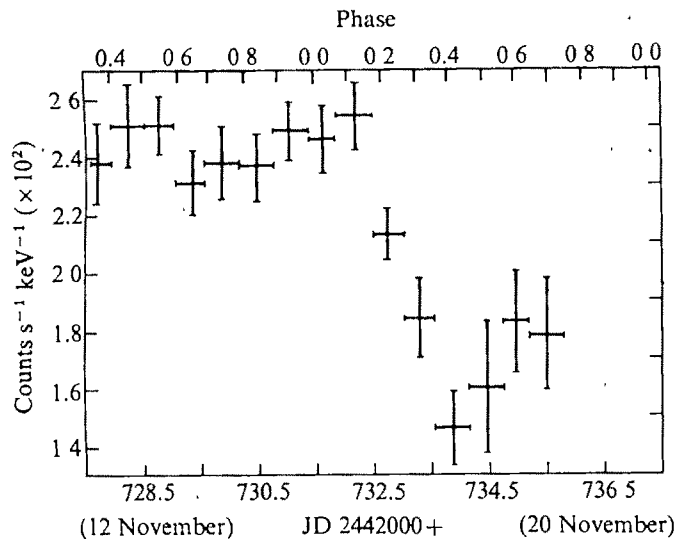


Fig. 1 The 23-153-keV counting rates observed from Cyg XR-1 in November 1975.

the 23-153-keV flux after the transition is still above that observed by us before the transition in 1975. Instead, it illustrates the large intrinsic variability present in the source even when in the 'low' state at lower X-ray energies. No 3 to 6 keV proportional counter data are available for comparison at the time of writing.

Typical spectra taken during periods of high and low intensity are shown in Fig. 3. The higher intensity spectrum, observed between phases 0.50 and 0.60 starting JD 2443092.505 (10 November 1976) is well fitted by a power law spectrum $dN/dE = C(E/E_0)^{-\alpha}$ photon $\text{cm}^{-2} \text{s}^{-1} \text{keV}^{-1}$ where $C = (1.8 \pm 0.1) \times 10^{-3}$ photon $\text{cm}^{-2} \text{s}^{-1} \text{keV}^{-1}$, $\alpha = 2.1 \pm 0.1$ and $E_0 = 73.6$ keV; the lower intensity spectrum, observed between phases 0.40 and 0.50 starting JD 2442733.583 (17 November 1975), is well fitted by a power law spectrum with $C = (1.0 \pm 0.2) \times 10^{-3}$ photon $\text{cm}^{-2} \text{s}^{-1} \text{keV}^{-1}$, $\alpha = 2.4 \pm 0.4$ and $E_0 = 57.2$ keV. The quoted uncertainties are 68% confidence intervals calculated according to the method outlined by Lampton *et al.*¹⁴. The anti-correlation between intensities at lower and higher energies indicates that the spectrum must pivot about a point between the two energy ranges. From the observations of Coe *et al.*¹³, this pivot point occurs at ~ 7 keV.

All individual spectra that we observe, measured over 0.6-d intervals, can be well fitted by a power law, although the

constant C varies from spectrum to spectrum. The resultant uncertainties in α obtained from fits in our limited energy range do not allow us to show a detailed correlation between observed spectral index and the 23-153-keV counting rate. All the 1976 data are at higher intensity than the 1975 data, however, and the 1976 data have a lower average value of α (2.05 ± 0.03) than the 1975 data ($\alpha = 2.39 \pm 0.04$), as would be expected from the discussion above. If these average spectra extend unchanged to lower energies, the 2-300-keV luminosity of the system is in fact greater when the source is more intense at lower energies (that is, the 'high' state at 3-6-keV is indeed the higher luminosity state of the system).

This pivoting behaviour (or 'see-saw' effect) is predicted to occur in models for which the X-ray emission is produced in a two-temperature accretion disk surrounding the secondary component of the binary system^{8,7}. In these models, the inner part of the accretion disk, ballooned up by radiation pressure, is geometrically thick but optically thin to higher energy X-rays. The high-energy radiation, predicted to have a power law spectrum, is produced from the lower energy X-ray or ultraviolet photons by inverse Compton scattering off relativistic electrons in this region of the disk. Basically, an increase in the lower energy photon flux 'cools off' the electrons by causing them to undergo more inverse Compton collisions, leading to a lower mean energy gained by the photons per scattering and, ultimately, to a lower flux of high energy photons. This anti-correlation between the fluxes in the two energy ranges would then be observed as the same type of pivoting of the spectrum that we detect in Cyg XR-1.

Transitions in Cyg XR-1 are observed to occur at irregular intervals, but seem to occupy much the same duration, about one day, regardless of direction of the transition or interval since the last transition¹⁻³. We note that not only the two transitions we observed (one of which may not be associated with a large change in the low energy flux) but also six of the seven other transitions in the intensity state of Cyg XR-1 reported in refs 1-5 commenced near periastron at phase 0.18 (ref. 10). It is tempting to speculate that transitions in the luminosity are linked to variations in the mass transfer rate from the primary to the accretion disk surrounding the secondary. Alme and Wilson¹⁵ have proposed a model of the system in which matter is transferred primarily by radiation-driven density waves generated in the atmosphere of the primary, which fills only about 85% of its Roche lobe. In such a model having the orbit eccentricity and period of the HDE 226868 system, nearly all the mass is transferred in an interval approximately 1 d long near periastron. As pointed out by Alme and Wilson, the period between mass transfer 'pulses'

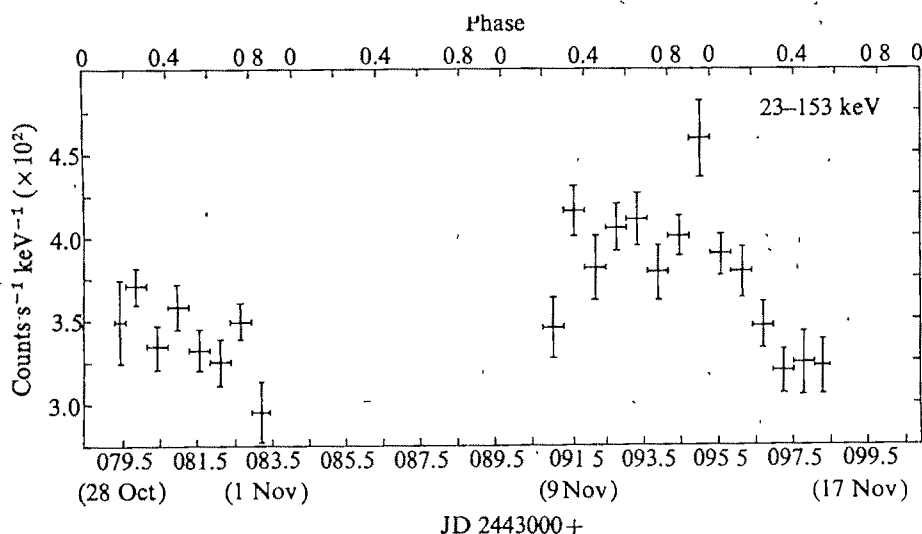


Fig. 2 The 23-153-keV counting rates observed from Cyg XR-1 in October and November 1976. Note the difference in the scale of the ordinate from Fig. 1. Spacecraft pointing requirements prevented observations between 1 and 7 November.

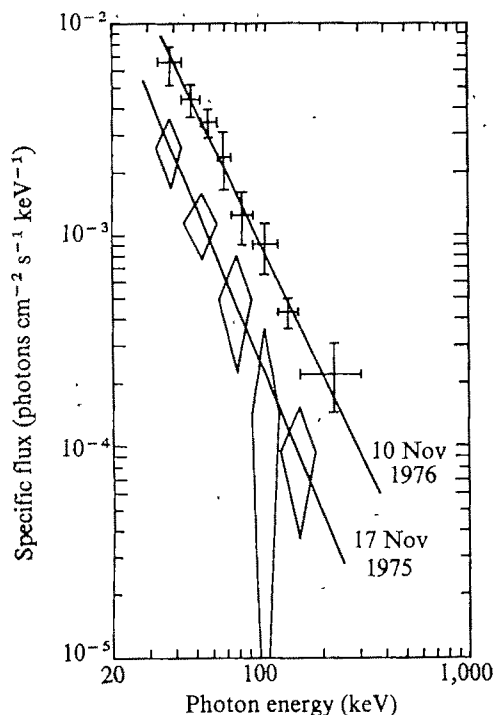


Fig. 3 Typical spectra taken during a 3-6 keV 'low' state (crosses, above), and a 'high' state (diamonds, below). The straight lines are power law fits to the spectra; spectral parameters are given in the text. Error bars are ± 1 standard deviation in the flux. Individual channels in the original data have been grouped to improve statistics.

is not critically phase locked and the period between pulses is small compared with the characteristic disk transfer time. Hence, variations in the X-ray emission at this period would be difficult to detect if approximately the same amount of mass were transferred every pulse. If the primary, for some unspecified reason, suddenly changed drastically the amount of matter it transferred per pulse, however, the disk might then be expected to show a changed luminosity commencing near periastron.

A large increase in mass transferred per pulse would presumably increase the low energy photon flux in the outer regions of the disk. The high energy flux would then follow in an anti-correlated fashion, as discussed above. The difference between 'transitions' and 'transient events'¹² would then be related to whether the mass transferred in succeeding pulses was maintained at its new value or returned to its previous level. It will be interesting to see whether this correlation of commencement time with phase near periastron is borne out in future transitions.

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On the association between a γ -ray burst and a radio pulse

ONE minute after the γ -ray burst of 16 August 1976, a radio event with ~ 30 s duration was recorded by three different instruments, a dipole array and a 10-m parabola at Medicina (Bologna), and a 10-m parabola at Trieste (400 km away) (refs 1 and 2). The peak intensity at 151 and 408 MHz was $\sim 10^{-21}$ W m $^{-2}$ Hz with an upper limit to the dispersion between the two frequencies of ~ 10 s. In the absence of specific data on the γ -ray flux we take a typical value of 10^{-4} erg cm $^{-2}$ corresponding to an energy output of 10^{38} d_{100}^2 erg, where d_{100} is the distance in units of 100 pc.

Here we shall assume that the γ - and radio pulses are physically associated and discuss some implications on the nature of the source. The duration and time lag of the radio pulse are reminiscent of flares in ultraviolet Ceti stars³ where the main activity is in the optical band. However the larger power and temperature required for γ -ray bursts favour models involving white dwarfs or neutron stars.

The brightness temperature of the radio pulse is

$$T_b = (3.2 \times 10^{11})/R^2 d_{100}^2 \text{ K} \quad (1)$$

for a source dimension $R \simeq 10^8$ cm and $d_{100} \simeq 1$, $T_b \simeq 10^{23}$ K — of the same order as found in pulsars. This implies a coherent emission mechanism. Assuming that this is synchrotron radiation, a number of constraints leading to an estimate of the relevant physical parameters can be arrived at. The synchrotron power is⁴ given by the expression

$$\frac{1e^2c^2}{2\pi c \rho^2} \gamma^4 n V N = 4.5 \times 10^{33} \alpha d_{100}^2 \text{ erg s}^{-1} \quad (2)$$

where the observed radio power has been introduced on the right side, α is the beaming factor ($\alpha = 1$ if the emission is isotropic), n is the density of relativistic particles with Lorentz factor γ , ρ is the curvature radius of the particle trajectories, V is the volume of the emitting region and N the coherence factor, which must satisfy the relation

$$N \geq KT_b/\gamma m_e c^2 \quad (3)$$

If the observation frequency is the critical synchrotron frequency one has

$$\nu_{cr} = \gamma^3 \frac{c}{2\pi\rho} = 4 \times 10^8 \text{ Hz} \quad (4)$$

Minimising the energy requirements we further impose that the relativistic particle energy is radiated in the radio band during the crossing time of the emission region R/c

$$\gamma m_e c^2 n V = 4.5 \times 10^{33} R/c \alpha d_{100}^2 \text{ erg} \quad (5)$$

Equations (1), (2), (3), (4) and (5) yield

$$N = 3 \times 10^{15} \gamma^3/R \quad (6)$$

$$N \geq 5.5 \times 10^{31} d_{100}^3/(\gamma R^2) \quad (7)$$

For ordinary synchrotron radiation in a dipole field an addi

tional relation is

$$\rho = \frac{\gamma m_e c}{3 \times 10^6 B_s} \left(\frac{R_s}{R_{s0}} \right)^3 \quad (8)$$

where $\gamma m_e c$ is the electron momentum in eV/c, θ is the pitch angle, and $B_{s0} = 10^{-6} B_s$ (G) is the surface magnetic field and $R_{s0} = 10^{-9} R_s$ (cm) is the radius of the star.

The system gives

$$R_s \geq 1.36 \times 10^3 \theta^{2/7} R_{s0}^{6/7} B_s^{2/7} d_{100}^{2/7} \quad (9a)$$

$$\gamma \geq 20 \theta^{-1/14} B_s^{-1/14} R_{s0}^{-2/14} d_{100}^{6/14} \quad (9b)$$

$$n \leq 10^4 \alpha \theta^{-0.5} B^{-0.5} R_s^{2/14} d_{100}^{10/7} \text{ cm}^{-3} \quad (9c)$$

In the limit where $\theta \rightarrow 0$, the radiation due to particles following the field lines reduces to 'curvature' radiation. For $\rho \simeq R$, one has

$$R_s \geq 4 \times 10^{-3} d_{100}^2 \quad (10a)$$

$$\gamma \geq 140 d_{100}^{2/3} \quad (10b)$$

$$n \leq 10^{10} \alpha d_{100}^{-2/3} \text{ cm}^{-3} \quad (10c)$$

The main difference between the two sets of relations is that in the former case even for a small pitch angle, $\theta \sim 10^{-2}$, a much larger extension is implied. Since it seems extremely difficult to produce and maintain coherence conditions over large scales we suggest that curvature radiation is responsible for the radio-emission. The associated synchrotron radiation is emitted at much higher frequency where presumably it is not amplified by coherence. This picture and the derived values of the parameters are similar to those proposed by Pacini and Rees for pulsars⁶.

Because of the upper limit on the signal dispersion it is not possible to explain the 1-m delay between the γ -ray and radio events as caused by the increasing transparency of an expanding cloud. The delay could be either intrinsic or due to a beaming of the radio emission and to rotation of the source. The period should be a few minutes and the total duration of the radio emission comparable. This could explain the previous negative observations⁶ because one expects that only for a small fraction of the γ -ray events the associated radio emission will be detectable at Earth.

If the underlying star is a neutron star the hypothesis that the delay is intrinsic is difficult to support since the Alfvén velocity is large. The rotational energy of a neutron star with $\omega \simeq 10^{-3}$ and its magnetic energy for $B \simeq 10^{12}$ G are both $\simeq 10^{41}$ erg. In the burst 10^{-3} of the available energy should be released and in both cases this should be accompanied by a pulsar glitch⁷. However the fractional energy variation required is much larger than the maximum observed period discontinuities in pulsars. A possible way out is the interaction with an external agent (for example, a companion star), which on the other hand, implies a serious difficulty in explaining the short timescale⁴.

Production of γ -ray bursts by magnetic white dwarfs has been proposed by some authors⁸⁻¹⁰. The mechanism should be a convective instability, which can account for the similarities with ordinary flares. In this model regions with magnetic fields $\sim 10^8$ - 10^9 G are required⁹, while the average surface field B_s can be lower and, for $B_s \sim 10^6$ G, the burst energy is comparable with the total magnetic energy. On the other hand, from the known density of magnetic white dwarfs in the sun vicinity, a burst frequency of 1/40 per year per star is required in order to account for the observed γ -ray event rate. Therefore either the density of magnetic white dwarfs has been underestimated by a large factor or one must suppose that a

dynamo mechanism can reconstruct the magnetic field at the expenses of the rotational energy, which for $\omega = 10^{-3}$ is 10^{47} erg.

Note added in proof: In a recent preprint by Sommer *et al.* serious doubts are raised on a direct association between the radio and γ -ray events of 16 August 1976.

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⁴⁰Ar-³⁹Ar age of rocks, and the development mode of the Philippine Sea

THE origin and development of the Philippine Sea have been central issues in tectonic studies of a marginal sea: the deep-sea drilling project (DSDP), Leg 31, was primarily intended to resolve the question¹. Unfortunately, at only two of the Leg 31 sites (292 and 296) were microfossils indicating the age of the basement recovered, so the age of the ocean basin, had to be deduced by dating the drilled basement rocks. There seems to be some confusion in the literature² in interpreting the fossil data with regard to the age of the ocean basin. We have dated several DSDP Leg 31 drilled rocks by the ⁴⁰Ar-³⁹Ar step-heating technique (Table 1 and Fig. 1) and re-evaluated the fossil ages and various hypotheses concerning the development of the western Philippine Sea and the Shikoku Basin. We applied two statistical criteria to judge the reliability of ⁴⁰Ar-³⁹Ar ages, (1) the experimental data with MSUM values (Table 1) less than 3.50 and (2) the apparent ages should agree with each other, within 1 σ , for more than 65% of ³⁹Ar released. Although such criteria do not prove the reliability of this dating technique, they provide a measure of it³. Of the nine drilling sites, six reached the basement—site 290, 291, 292, 293, 294 and 296—their ages were determined by both the ⁴⁰Ar-³⁹Ar step-heating dating and micropaleontological methods (Table 1).

Earlier views as to the development of the Philippine Sea suggested that the western Philippine Basin is Mesozoic or even older, and the CBF was an extinct oceanic ridge^{4,5}. On the basis of the fossil ages obtained from Leg 31, Karig *et al.*⁶ proposed that the Philippine Basin was a complex early Tertiary basin developed at least in part behind an pre-existing arc system. Loudon⁷ identified ESE-WNW trending magnetic lineations south of the Central Basin Fault as anomalies 18 through 21. Recently, Watts *et al.*⁸ reported ESE-WNW trending lineation north of the CBF, which they suggested as either Mesozoic or Tertiary in age, although preferring the latter. However, there are no Mesozoic basement ages in the western Philippine Sea and no age progression with distance from the CBF, which would be expected if the CBF is an extinct spreading ridge (Fig. 1).

There seems to be considerable difference between the ⁴⁰Ar-³⁹Ar age and microfossil age for site 292. The lowermost clastic rock (chalk: core 39-section 1-88-99) can be identified to belong to P 15 of Blow's planktonic foraminifera zonation scheme⁹ in the late Eocene. If a timescale of Berggren¹⁰, and Berggren and Van Couvering¹¹ is

Table 1 Age determination of drilled basement rock

Sample	Rocks	Total age† (Myr)	Plateau age‡ (Myr)	⁴⁰ Ar- ³⁹ Ar ages* Isochron ages (Myr) and J-value	MSUM = $\left(\frac{\sum (f_i - \bar{f})^2}{n-1}\right)^{1/2}$ for all fractions but 700 °C	Best age for the ocean basin (Myr)
DSDP 292-41-2	coarse grained basalt	51.1	49.4 ± 1.5 (for 95% ³⁹ Ar)	49.5 ± 2.0 (J = 0.006955)	1.208	49.4
DSDP 293-21-2	gabbro, cumulate rock	440.7	42.0 (73%)	42.0 (J = 0.006955)		42.0
DSDP 294-7-1	alkali basalt	33.9	49.1 ± 3.5 (95%)	48.8 ± 2.0 (J = 0.006955)	1.173	49.0
DSDP 296-63-4	lapilli tuff	51.8	47.9 ± 4.0 (72%)	47.5 ± 4.0 (J = 0.010201)	1.377 for all fractions	47.5

* $\lambda_e = 0.585 \times 10^{-10} \text{ yr}^{-1}$, $\lambda = 5.305 \times 10^{-10}$, $^{40}\text{K}/^{39}\text{K} = 1.19 \times 10^{-4}$

†Total age = $(1/\lambda) \ln \{1 + J(^{40}\text{Ar}-^{39}\text{Ar})_{\text{total}}\}$, where $(^{40}\text{Ar}-^{39}\text{Ar})_{\text{total}} = \sum f_i(^{40}\text{Ar}-^{39}\text{Ar})_i$, f_i = fraction of ³⁹Ar released.

‡Defined for the fraction of ³⁹Ar released, which is indicated in a bracket.

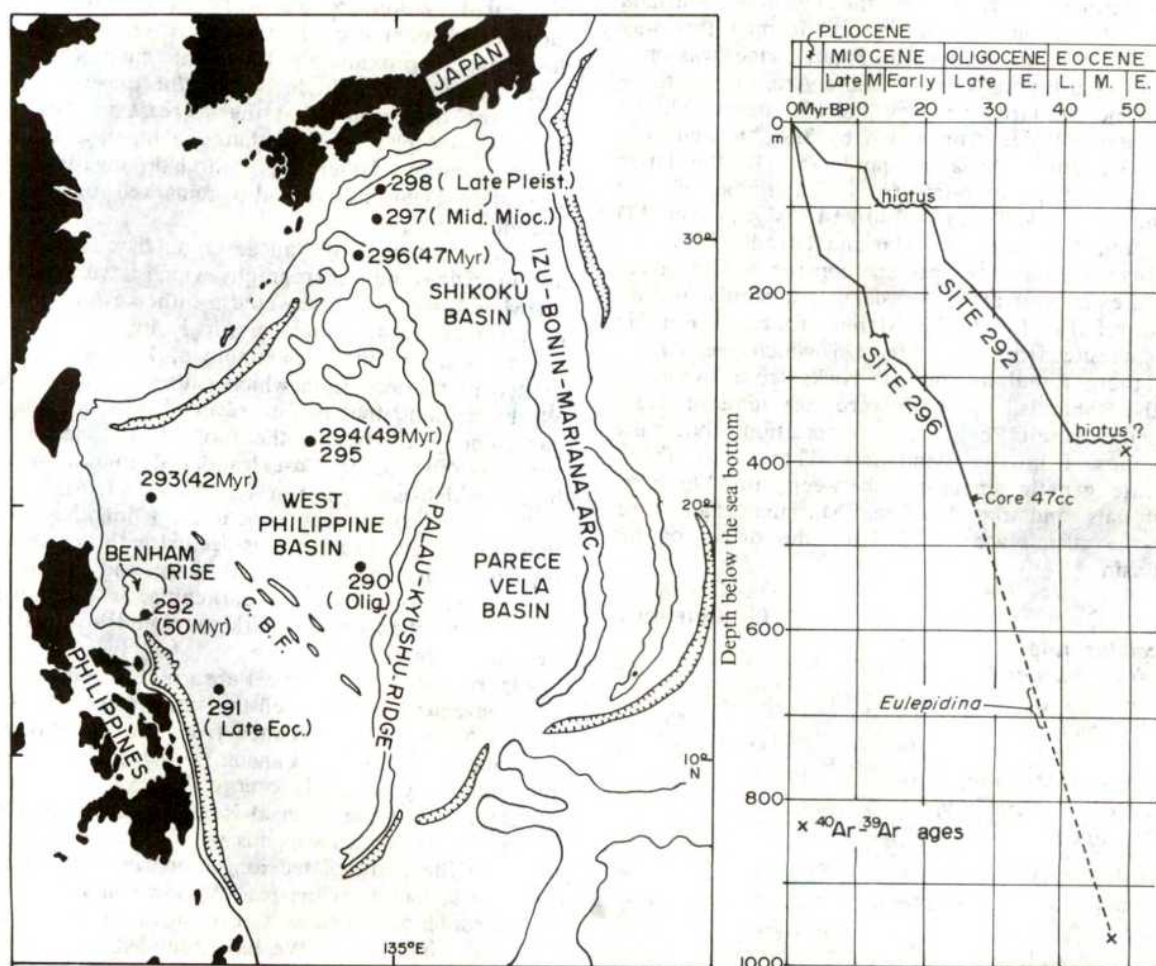
§Determined for the experimental data (temperature fractions) by means of York's least square fitting method.

||The isochron and plateau ages were determined only for three lowest temperature fractions; 600, 700, 800 °C. Hence, the age may not be very reliable.

adopted, the age would be approximately 41 Myr BP. An ⁴⁰Ar-³⁹Ar age obtained for a basalt (about 16 m below the chalk sample) gives 47.5 Myr BP (Fig. 1) although this age difference may be due to a depositional hiatus. A K-Ar age obtained by McKee¹² for Core 45 (38.1 Myr BP) and Core 47 (37.1 Myr BP) may reflect Ar-loss or some alteration effects, since the K-Ar ages are even younger than the microfossil ages in the overlying sediments (Core 41) which is about 40 cm above the basalts. Although nannofossils,

were tentatively assigned the lower Pliocene age for the basalt part of the Hole 293, their rarity means that the data are highly questionable. As the basalt sample used for ⁴⁰Ar-³⁹Ar dating is brecciated gabbro¹, the radiometric age is unlikely to represent the basement age. Hole 294 is the northernmost drilling site in Philippine Sea and also furthest from the CBF. If the CBF was truly a spreading ridge axis, one would expect to find the oldest age at this site, but this is not the case.

Fig 1 a, DSDP Leg 31 sampling sites; ⁴⁰Ar-³⁹Ar ages (Myr) and minimum basement ages estimated by microfossils are shown in a bracket (left). b, the fossil dates and ⁴⁰Ar-³⁹Ar ages are plotted against a depth below the sea bottom for site 292 and 296, which are only sites in Leg 31 to show a reliable basement fossil age.



Summarising, the western Philippine Basin is very likely to be Tertiary in age, there is no positive age evidence to suggest the spreading ocean ridge origin of CBF and the Philippine Basin is significantly younger than the currently proposed magnetic anomaly lineations would imply⁴⁻⁷.

Several authors¹²⁻¹⁵ have identified magnetic anomaly lineations in Shikoku Basin suggesting a spreading origin for the Basin. Using Leg 31 data, Watts and Weissel¹⁶ estimated the oldest age of the basement to be 24 to 27 Myr BP, and that the east-west-directed spreading of Shikoku Basin started about the same time. Only one ⁴⁰Ar-³⁹Ar age has been obtained for lapilli tuff at site 296, located on the Kyushu-Palau Ridge, and this is considerably older than the assumed basement age¹⁶. Site 296 shows gradational change from purely lapilli tuff to pure clastic rock (mainly nannofossil chalk); in Core 38, *Eulepidina*, a larger foraminiferan, indicated shallow water habitat around the middle (Core 56 and 57) of this changing interval¹. These results suggest that the pyroclastics accumulated in a rather shallow sea which then subsided. An average sedimentation rate of about 2.72 cm per 1,000 yr can be estimated for the time interval from 22.5 Myr BP (M/O boundary detected by planktonic foraminifera) to 47.5 Myr BP (⁴⁰Ar-³⁹Ar age) (Fig. 1) which is significantly faster than that of the overlying sediments. The ⁴⁰Ar-³⁹Ar age result is, therefore, in accordance with that expected from the sedimentation rate. The difference between the ⁴⁰Ar-³⁹Ar age and the assumed basement age indicates that either the opening of the Shikoku Basin was a much later event than the formation of the Kyushu-Palau Ridge, or, that the identification of the magnetic anomaly lineation is in error. To resolve the question more radiometric ages of the basement rocks in Shikoku Basin are needed.

The ⁴⁰Ar-³⁹Ar age of the lapilli tuff, seems to favour a suggestion¹²⁻¹⁶ that Shikoku Basin, as well as Parece Vella Basin, was formed by rifting of the Izu-Bonin-Mariana Islands Arc. If the Shikoku Basin was formed this way DSDP site 296, now on the Kyushu-Palau Ridge, was once located on the Izu-Bonin-Mariana Island Arc, or else there should be some similarity between the basement rocks of the Kyushu-Palau Ridge represented by Site 296 materials and of the Izu-Bonin-Mariana Island Arc. In the latter augite-orthopyroxene andesite from Hahajima of the Bonin islands gave a K-Ar age of 40.1 ± 1.2 Myr BP (ref. 17) and dacite from Saipan of the Mariana Islands yielded an age of 45 Myr BP. Both the ages are consistent with larger foraminifera evidence from these islands and similar to that for site 296 (ref. 18). In addition Meijer¹⁹ found Pb isotopic ratios in andesite from the Site 296 which were quite similar to those found in volcanic rocks from Iwo-Jima, south of the Bonin Islands, both were very different from typical oceanic basalt Pb isotopic compositions, but were similar to those from the island arcs. These facts, therefore, indicate genetic similarities between site 296 basement materials and the Izu-Bonin-Mariana island arc, favouring the rifting apart theory of the origin of the Shikoku Basin.

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Alpha-induced coloration reversal in biotite

Using previously developed techniques for the investigation of radiation coloration in biotite, we have discovered the hitherto unknown fact that the onset of reversal occurs precisely at the end of the α range and is a phenomenon independent of the darkening peak.

Since the earliest original investigations of Joly¹ into the nature of pleochroic haloes, it has been known that α radiation can produce a 'reversal' effect in biotite (sometimes called 'inversion'). The usual effect of natural or artificial α irradiation is to produce a characteristic darkening of the biotite. This darkening increases with dose until a certain point (that is, the optical absorption coefficient saturates) and then the mineral begins to lighten as the dose is further increased. With sufficient dosage it is possible to lighten the biotite to the point where it actually becomes less dark than its naturally occurring shade.

The most quantitative study of reversal was carried out by Deutsch^{2,3} and his collaborators. Their technique involved the irradiation of small flakes of biotite with non-collimated radon α particles. They found that in their experimental configuration reversal took place at doses over approximately 10^{17} alphas cm^{-2} which is about four orders of magnitude beyond the onset of darkening. They also determined that the decrease in darkening near saturation. Other important halo investigators before and after Deutsch have also remarked on the reversal phenomenon^{4,5}.

Using techniques of Van de Graaff irradiation of biotite and precision photomicrography which have been described elsewhere⁶, we have discovered a hitherto unknown feature of reversal. In the literature it is implicit that reversal occurs at the point of maximum darkening or, at least, is related to the ionisation which causes the darkening peak. We have found that in fact reversal does not begin at the darkening peak, but at the foot of the peak at a point corresponding to the α -extrapolated ionisation range in biotite which is $35.1 \mu\text{m}$ at 7.69 MeV (ref. 6).

The most significant conclusion from these results is that the reversal process is associated with the depositing of α -particles at the end of their range and is not an effect due to ionisation, as is the darkening. In other words, the physical mechanisms of darkening and reversal seem to be quite different.

Figure 1 shows the optical absorption coefficient for four successive α -irradiations of the same piece of biotite. (As in ref. 6 we used a specimen from the Faraday pegmatite in Bancroft, Ontario, Canada.) For clarity we used the maximum ²³⁸U α -family energy, 7.69 MeV. The successive development of the reversal is readily apparent. The minimum of reversal corresponds within the error of measurement to the extrapolated ionisation range as given above. Our work, based on the results shown in Fig. 1 and other bombardments, indicates a reversal onset at approximately 5.2×10^{14} alphas cm^{-2} . We have found a plateau area of at

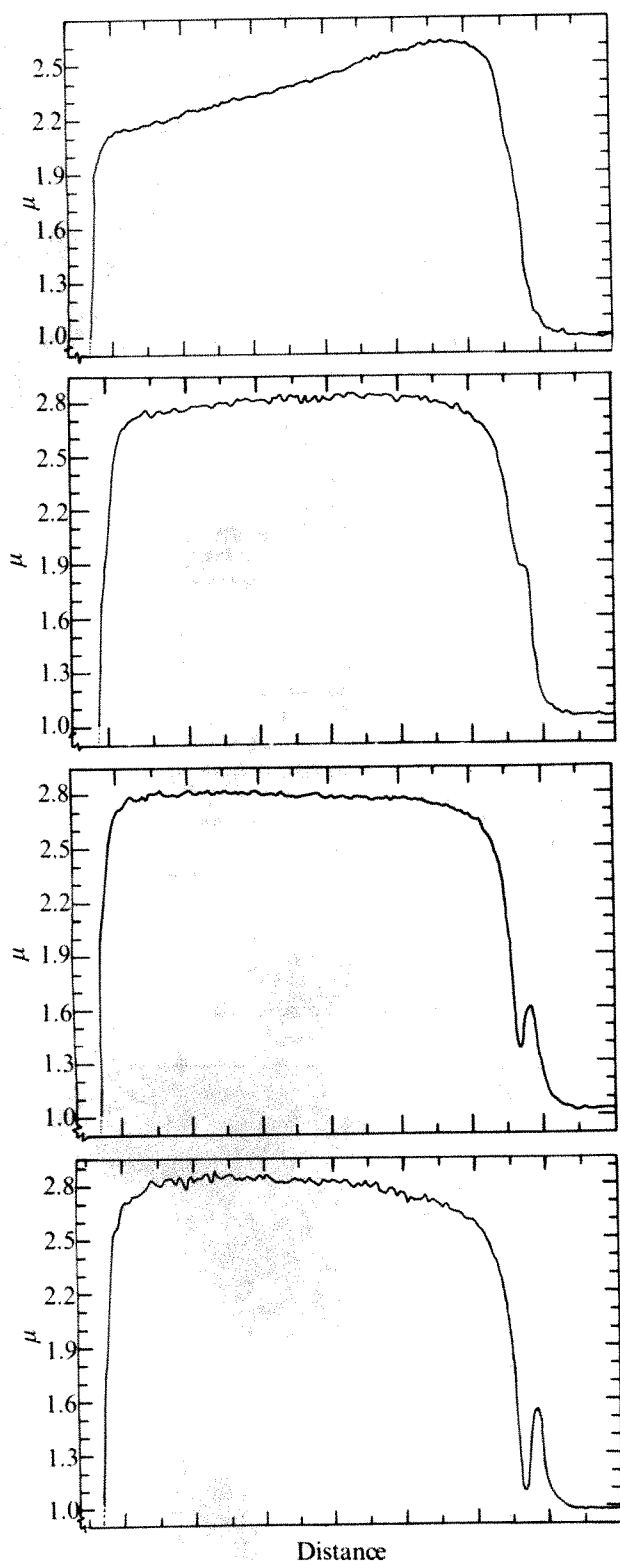


Fig. 1 These curves are densitometer tracings, suitably processed according to the method of ref. 6, of single-energy α bombardments of biotite. For each curve the ordinate is the optical absorption coefficient (in units of the natural coefficient for the biotite so that $\mu = 1$ is the unirradiated mineral). All four energies were 7.69 MeV and the doses, from top to bottom were approximately 2.6×10^{14} alphas cm^{-2} , 10^{15} alphas cm^{-2} , 2.1×10^{15} alphas cm^{-2} , and 4.0×10^{15} alphas cm^{-2} . Note the distinct separation between the maximum coloration at $32.2 \pm 0.2 \mu\text{m}$, and the reversal at the extrapolated ionisation range of $35.1 \pm 0.2 \mu\text{m}$.

most one order of magnitude between saturation and the onset of reversal. Our onset of reversal dose is several orders of magnitude below that found by Deutsch and our plateau area somewhat smaller. We do not consider that these conflict with Deutsch's results considering the precision of our work and his semi-quantitative approach. We repeat, however, that the reversal onset does not occur at the same place along the α track as does the darkening peak. The latter occurs at the point of maximum ionisation⁶.

Previous explanations of reversal go back to Joly who believed the halo development process to be a photographic-like one with a latent image being 'developed' by the α radiation. He attributed the reversal to some unspecified 'photographic' effect. Henderson and Turnbull⁴ made Joly's conjectures more specific by attributing the reversal to an Eberhard-like effect in the biotite. In the development of a negative the Eberhard effect causes regions adjacent to sharply defined dense areas to become lighter than they should due to a diffusion into the affected regions of an excess of soluble bromide. This misinterpretation of the Eberhard effect caused Henderson and Turnbull to underestimate the alpha ranges when determined from reversal haloes⁴. As our Fig. 1 shows, the reversal effect has a distinct and sharply defined onset and is not at all characteristic of an effect diffusing from the darkening peak outward.

Deutsch and Janssens also considered the geological phenomenon of metamictisation and concluded after some simple calculations that this could not be the cause of reversal.

The phenomenon of reversal does not seem well known in solid state physics. It would be of interest to α -bombard some well understood system, such as an alkali halide, to see if colour reversal could be obtained.

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The Thera eruption and Late Minoan-IB destructions on Crete

MARINATOS' theory¹ of the decline of the Minoan civilisation by paroxysmal volcanic events of the Thera volcano is widely accepted, and many papers on the topic have been published, especially since the International Scientific Congress on the Volcano of Thera². The main and controversial problem is the fact that the simultaneous devastations of numerous Late Minoan (LM) IB sites and palaces in Crete are dated archaeologically (concerning the development of Minoan pottery) as 30-50 yr later than the destruction of the important LM settlement on Santorini (Acrotiri ruins)³. Following Marinatos' theory, that the devastations in Crete were caused by the Thera volcano, two eruptions (if

chronology is correct) must have occurred. The first, destroying the LM-IA settlements on Santorini, in about 1500 BC, the second, causing the LM-IB destructions in Crete, about 1450 BC. According to a recent theory, only LM-IA destructions on Crete are connected with the Thera outburst^{4,5}. We report here fieldwork on Santorini which shows clearly that neither products of two volcanic outbursts nor of a 'two-phase' eruption⁶, differing in a time interval of about 50 yr, exist, and that the collapse of the Santorini caldera, which caused devastating earthquakes and tsunamis in Crete, did not occur about 50 yr after the volcanic paroxysm.

If two eruptions had occurred, volcanic products of both would exist. In that case, the time interval between both volcanic events should be manifested by an erosional disconformity and a weathered layer dividing the products of the two eruptions. From the volcanological point of view, the experts are unanimous that the Upper Pumice Series⁷, which covers the destroyed LM settlements on Santorini, was deposited during a single volcanic phase. We carried out new fieldwork on Santorini in autumn 1976 which confirmed this current view. According to the stratigraphy and lithologic character of the Upper Pumice Series⁸ the LM outburst began with catastrophic earthquakes that, after an interval between some weeks and 2 yr (ref. 2), were followed by a large pumice-fall. This pumice-fall layer is overlain by base surge deposits⁹, which locally show a slight erosional break. It should be stressed, however, that this break represents only a very short span of time of volcanic inactivity, that is of some days up to at most few weeks. The eruption culminated in some violent ash-flow eruptions by which the bulk of the Upper Pumice Series was deposited. These eruptions were connected with the beginning of the collapse of the central part of the old

Santorini island. This is evidenced by abundant huge lithic blocks, as much as 3 m in diameter, embedded in the ash-flow material. These blocks were formed by a falling-in of parts of the old volcanic pile along circular and/or polygonal fractures. Still in the course of the collapse the blocks were ejected immediately by simultaneous paroxysmal eruptions. This evidence is inconsistent with Doumas' theory¹⁰ of a formation of the Santorini caldera about 50 yr after the Thera outburst, that is around 1450 BC. This theory should explain the time interval between the devastations both on Santorini and in Crete.

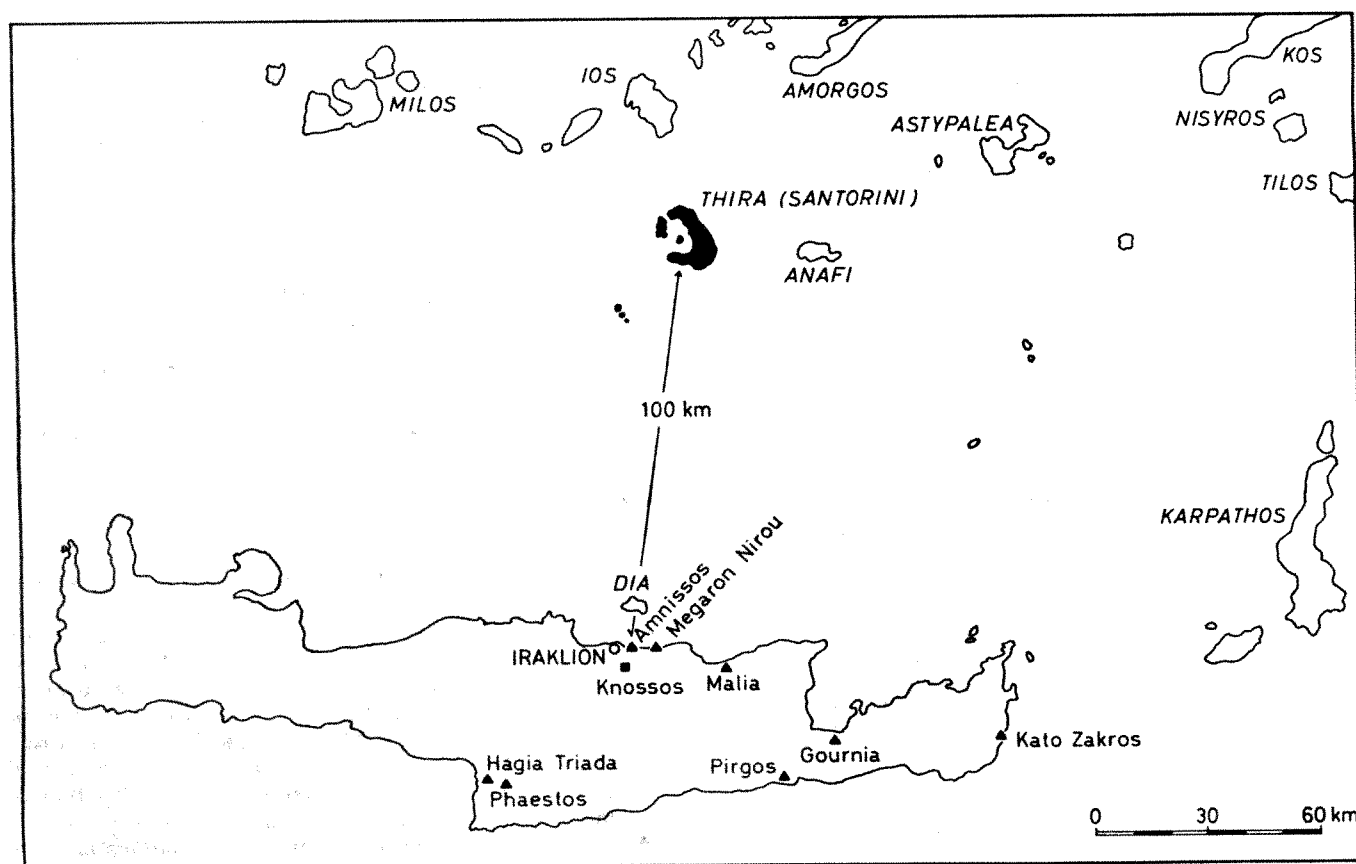
According to our investigations on Santorini and Crete, the devastations in Crete around 1450 BC can be explained only by the following alternative. (1) The archaeological dates must be revised. The destructions on Santorini and in Crete occurred simultaneously (both around 1500 BC or both around 1450 BC).

From the archaeological point of view, a modification of the ceramic chronology is not supported and would, moreover, involve insuperable difficulties. (2) The devastations in Crete were caused neither by the eruptions of the Thera volcano nor as a result of the subsidence of the Santorini caldera.

The results of our fieldwork are inconsistent with the theory that the LM-IB devastations in Crete were the consequence of a volcanic disaster on Thera. According to this theory^{1,11,12,13,14} the LM-IB devastations on Crete have been caused by tephra-fall and/or tsunamis and/or catastrophic earthquakes, connected with the Thera outburst or the Santorini caldera collapse.

Tephra-fall: Our fieldwork showed that visible remnants of the LM tephra-layer in central and eastern Crete do not exist. Special attention was paid to localities in which such remnants should have been preserved, for example in

Fig. 1 The most important Late Minoan sites on Crete and the location of Thira (Santorini) and other Cycladian islands.



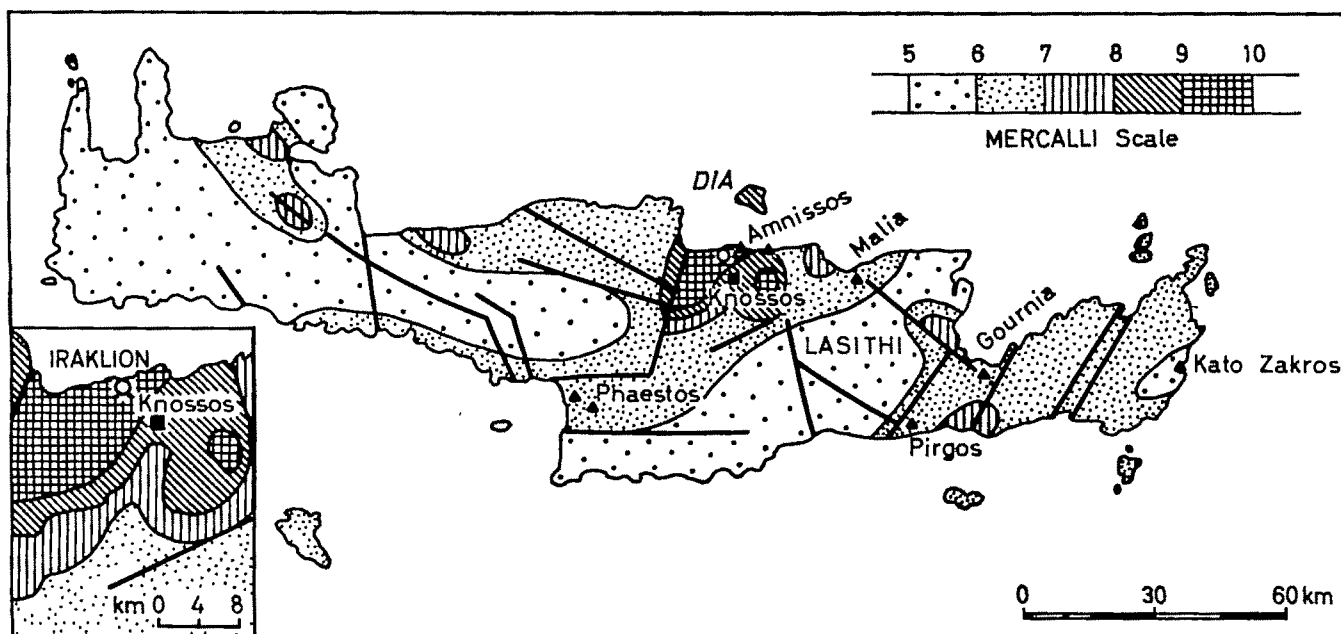


Fig. 2 Isoseismic sketch map of Crete of the great regional earthquake from June, 26, 1926. The relation of the earthquake intensity to the block structure of Crete is clearly visible. (According to Sieberg²¹.)

recent soil profiles at the northern coast, exposed by deep open cuts and drain channels or in crevices and sinks in the limestone mountains around the Lasithi plateau. Residues of some washed soil samples (from Pargos and Malia) contain few small grains of volcanic origin, that is, feldspar, volcanic glass ($n \sim 1.510$, which corresponds to the Upper Pumice Series on Thera⁸), and rare pyroxene. Their meagre quantity, however, is inconsistent with a suggested thickness of the tephra-layer of up to 10 cm (ref. 6) or even of 1 cm (ref. 15). Quantities of 50–750 volcanic particles per cm^3 as indicated by Boekschoten¹⁴, have never been found. The discrepancy between the refractive index of the glass shards in Boekschoten's samples, that is, $n \sim 1.503$, and the index of the volcanic glass of the Upper Pumice Series ($n \sim 1.510$, ref. 8), was already stated¹⁴. In other publications dealing with the presence of LM volcanic tephra on Crete^{16,17} is reported that only traces of such tephra have been found, the highest concentrations only in Minoan settlements.

There is a simple explanation for such a presence of pumice particles in these settlements, namely the use of pumice in workshops and in households. Lumps of pumice were already used by Minoan people for various purposes. This is illustrated by findings of numerous lumps and blocks of pumice (up to 30 cm in diameter) in Malia, Gournia, Knossos and Kato Zakros. Because of its size this pumice could not have reached Crete by way of an even paroxysmal volcanic eruption on Santorini, Milos or Nisyros. This material was mainly imported from these islands. Pieces from Malia, Knossos and Kato Zakros are characterised by grooves and traces of working.

The volcanic ash horizons in eastern Mediterranean cores, which were attributed to the Thera outburst and considered as an evidence of an important ash-fall in Crete and the surrounding Aegean Sea¹¹, are of three or four different ages¹⁸. The abnormal local thickness (as much as 212 cm) (ref. 11) of ash-layers in some cores is due to post-depositional mixing and transport. Furthermore, new problems concerning the stratigraphic correlation of these ash beds in the eastern Mediterranean cores have been pointed out¹⁹. The distribution chart of the tephra from the LM

Thera outburst, "imaginative but very uncritically"¹⁸ constructed by Ninkovich and Heezen¹¹, must therefore be discarded as one of the chief arguments for a disastrous tephra-fall on Crete.

Tsunamis: According to our observations in Amnisos, Megaron Nirou, Malia, Gournia and Kato Zakros the force of tsunamis originated by volcanic or seismic events in connection with the Thera outburst must have been more shallow than previously supposed¹⁴. According to our calculations, the uppermost height of the tsunamis amounts to only 8–10 m (ref. 20).

Volcanic earthquakes: The devastations in Crete about 1450 BC could not have been caused by volcanic earthquakes because the Thera outburst occurred about 50 yr earlier.

We conclude from the above that all non-human devastations around 1450 BC in Crete, that is, that of Amnisos, Megaron Nirou, Malia, Gournia, Kato Zakros on the northern and eastern coast and that of Pargos, Phaistos and Hagia Triada on or near the southern coast of Crete (Fig. 1)²⁰, have been caused by a catastrophic regional earthquake, similar to that of AD 1926 (refs 14, 21). The complicated block structure of Crete (Fig. 2) is the reason that, as during the great recent earthquakes (1926, 1856, 1846, 1815, 1780 and so on)²¹, tectonically unstable regions have violently been affected. More stable regions were only slightly touched. This regionally different behaviour of Crete with respect to earthquakes is consistent with the fact that the palace of Knossos was not demolished during the regional earthquake around 1450 BC. The same phenomenon was observed in 1926, when Evan's reconstructions of Knossos did not suffer but the neighbouring Iraklion was strongly demolished.

According to our observations there is no connection between the decline of the Minoan civilisation and the Thera outburst. This volcanic paroxysm occurred around 1500 BC and did not have a revival around 1450 BC, at a time of the great devastations in Crete. All speculations that the Thera outburst should have caused 'a large natural catastrophe in three stages', that is the plagues of Egypt, the deluge of Deukalion and the submergence of Atlantis¹⁴

are imaginative but, from the scientific point of view, not convincing.

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Charge stabilisation of very small particles of mixed oxides

IN our laboratory, a rock salt type structure has been found for mixed oxides of the type $Me(II)_xCr(III)_{2/3(1-x)}O$. $Me(II)$ can be Co, Ni, Cu, and Zn or mixtures of these. Other authors^{1,2} have found similar compounds of the general formula $Me(II)_xMe(III)_{2/3(1-x)}O$. We suggest a structure where a very small particle, of the order of 2–10 nm, has been stabilised by a surplus of O^{2-} ions on the surface, compensating an excess positive charge uniformly distributed in the bulk.

Besides the presence of $Me(II)_xCr(III)_{2/3(1-x)}O$, X-ray analysis shows MeO in varying small amounts. The particle size, as determined by X-ray diffraction, is typically in the order of 3–10 nm. In addition, there is a size dependence of the lattice parameter such that a small particle will have a relatively smaller lattice parameter of 412 pm, whereas for a 10 nm particle it will be around 420 pm.

From the structural data, one may conclude that the cations are randomly distributed on the octahedral sites in the crystal. Any ordered arrangements of these, or occupation of other sites, would destroy the rock salt structure. From electron diffraction and microscopy, it seems that in particular the (111) face is towards the vacuum side. In the zinc case, epitaxial ZnO (001) on $Zn_xCr_{2/3(1-x)}O$ has been observed (Fig. 1). Below 400 °C, the structure is stable in wet and dry atmospheres. Above this temperature, spinel phase and/or MeO/Cr_2O_3 is formed in an irreversible process.

As a possible explanation of these observations, we suggest a model where the particle surface has become negatively charged. If we, for simplicity, consider a finite linear chain of Me^{2+} , O^{2-} ions, the energy per O^{2-} ion would be lower than in the corresponding infinite chain. Substitution of 2 Cr^{3+} for 3 Me^{2+} and rearrangement of the chain so that both endpoints are occupied by O^{2-} ions results in a more stable configuration where the energy per O^{2-} ion is higher than in the infinite. The energy depends inversely on the chain length. For a bulk crystal with rock salt structure we may perform a similar process. Let us consider a small cube of NiO with an edge

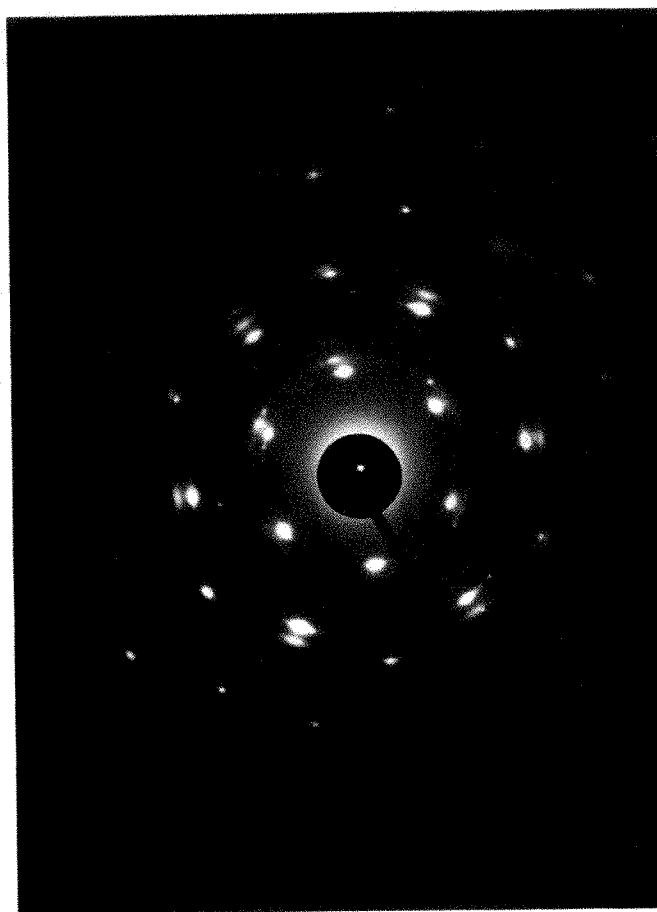


Fig. 1 Transmission electron diffraction patterns of a mixture of ZnO and $Zn_xCr_{2/3(1-x)}O$. The intense 6-fold symmetry spots arise from the (001) plane in ZnO while the less intense 6-fold symmetry spots are due to the (111) planes of the mixed oxide phase.

length of $\sim na$, where a is the lattice parameter ($= 418$ pm). The cube contains $4n^3$ NiO pairs; $12n^2$ of the sears located on the surface, edges and corners of the cube. Substitution of 2 Cr^{3+} in this lattice for 3 Ni^{2+} creates one cation vacancy. The surface acts as a sink for the vacancy and eventually 1 Ni^{2+} will be removed from the surface resulting in a negative charge of $-2e$ on the surface. It should be added that the Madelung energy of the Ni^{2+} is higher in the bulk than on the surface, making this process favourable. Assuming that the process is favourable as long as there are cations left on the surface, we end up with a crystal with

$$\begin{aligned} 4n^3 O^{2-} \\ 24n^2 Cr^{3+} \\ 4n^3 - 32n^2 Ni^{2+} \end{aligned}$$

$$so \quad x = 1 - \frac{9}{n}$$

For the size mentioned (~ 5 nm), n is about 12 corresponding to $x \approx 0.25$. Thus for small n the Cr^{3+} concentration becomes appreciable. On the other hand, this will result in a smaller lattice parameter, since the ion radius of Cr^{3+} is less than the ion radius of Me^{2+} . Two other energy contributions may stabilise this arrangement. Firstly, the smaller lattice parameter will result in a larger Madelung energy. Secondly, the Cr^{3+} ion has a sizeable octahedral configuration energy.

In the case of an octahedron, the surface-to-bulk ratio is higher than for the cube. Besides, we may construct the crystal so that it has the negatively charged (111) planes towards the vacuum side. Electron diffraction indicates that this is a likely arrangement (Fig. 1).

In order to estimate the energy changes in such an arrangement one should calculate, in addition to the Madelung energy, the attractive energy from the bulk surface interaction, as well as the repulsive energies from the negatively charged surface and from the positively charged bulk. This is not easily done, for a cube or for an octahedron. An estimate of the relative magnitudes involved, however, can be obtained by considering a 'spherical crystal' where the excess positive charge in the bulk is compensated for by a corresponding negative charge on the surface. We are not, in this argument, considering the repulsive potential between nearest neighbour ions, since this should be relatively independent of the various configurations we are considering.

Let N be the number of O^{2-} ions. The maximum number of O^{2-} in the surface is then given by $kN^{2/3}$.

We then have $2kN^{2/3}Cr^{3+}$ ions and $N-3kN^{2/3}Ni^{2+}$ ions. The surface has a total negative charge of $-2kN^{2/3}e = -Q$. Similarly, the bulk has an excess positive charge of $+Q$.

Using the superposition principle, one may calculate the electrostatic energies involved. Firstly, there is a Madelung energy from the lattice, being approximately

$$U_M = -\frac{\alpha}{a} \frac{1}{4\pi\epsilon_0} 4e^2 \left\{ \left(N - 3kN^{2/3} \right) + \frac{3}{2} 2kN^{2/3} \right\} \\ = -\frac{4\alpha}{a} \frac{e^2}{4\pi\epsilon_0} N \quad (1)$$

where $\alpha \approx 3.50$ is the Madelung constant and the first term represents MeO , the second (approximately) Cr_2O_3 .

Furthermore, there are the Coulomb energies from this particular charge arrangement.

$$U_C \approx \frac{Q^2}{4\pi\epsilon_0 r} \left(\frac{1}{2} + \frac{3}{5\epsilon} - \frac{1}{\epsilon} \right) = \frac{Q}{4\pi\epsilon_0 r} \left(\frac{1}{2} - \frac{2}{5\epsilon} \right) \quad (2)$$

the three terms in the bracket representing the surface repulsive energy, the bulk repulsive energy, and the attractive energy from the surface bulk interaction; ϵ is the dielectric constant. Inserting the value of Q and using $r = k_1 N^{1/3} a$, we find

$$U_C = \frac{k^2 N^{4/3} e^2}{4\pi\epsilon_0 r} \left(\frac{1}{2} - \frac{2}{5\epsilon} \right) \approx \frac{2.6 k^2 N}{4\pi\epsilon_0 a} e^2 \left(\frac{1}{2} - \frac{2}{5\epsilon} \right) \quad (3)$$

The maximum value of k is of the order three, whereas values of ϵ for very small particles are not known, but are most likely to be greater than one. The Coulomb term as such is then positive. In the limit for small spheres $\epsilon \rightarrow 1$ and

$$U_C \approx \frac{k^2}{4} \frac{Ne^2}{4\pi\epsilon_0 a} \quad (4)$$

The stability requirement is that the pair energy should be lower than for a pair in a cube of a similar size. For pure MeO , this is the stable configuration. The most stable configuration is obtained by minimising the expression for the total energy per O^{2-} ion with respect to N and k . Madelung energies of small cubes have been calculated by Christov³. Here we will use the approximative expression

$$U_0 \approx -\frac{4\alpha}{a_0} \frac{e^2}{4\pi\epsilon_0} \left\{ N - 0.24 N^{2/3} \right\} \quad (5)$$

where a_0 is the lattice parameter of pure MeO , and the last term in the bracket represents the smaller energy of surface and edge pairs.

A strongly charged surface will bind polar molecules such as water very strongly. A bonding energy of 200 kJ mol^{-1} will contribute about $-0.75kN^{2/3}e^2/4\pi\epsilon_0 a$

Including this, we obtain for the energy in the case of $k = 2$

$$U \approx \frac{-e^2}{4\pi\epsilon_0 a} N(14 - 1.0 + 1.5 N^{-1/3}) \quad (6)$$

The stability region is determined by $U/U_0 \geq 1$.

$$\text{Thus} \quad \frac{a_0}{a} \frac{13.0 + 1.5N^{-1/3}}{14 - 3.4N^{-1/3}} \geq 1 \quad (7)$$

The ratio a_0/a is a function of N . It represents the gain in Madelung energy by contraction of the lattice. Using Vegard's law and suitable values for ion radii we obtain in the present approximation $N \leq 1000$.

For $k = 1$, we obtain $N \leq 30,000$. If, instead, we used the pair energy of the infinite crystal as a stability limit, we would obtain $N \leq 600$ for $k = 1$. Thus, the model qualitatively explains the relative stability of small particles of mixed oxides. A similar situation could exist for other systems. It might also explain the growth of octahedral crystals of $NaCl$ and KCl under various circumstances.

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State of iron in glass-like carbon prepared by heat treatment of ferrocene resin

We have investigated the conversion of organometallic polymers to inorganic materials by heat-treatment. Several of those inorganic materials are industrially useful. Earlier studies have been reported in this journal^{1–3}. We describe here the state of iron dispersed in a glass-like carbon, which was prepared by heat-treatment of an organometallic resin containing ferrocene skeletons as the main chain. This state is unusual and strange both physically and chemically.

The organometallic resin was synthesised by the polycondensation of acetylferrocene and furfural using sulphuric acid as the catalyst⁴. When the molar ratio of furfural (F) and acetylferrocene (A) exceeded $F/A = 15$, the resin was not formed. Acetone was added to the starting material which contained furfural at an F/A ratio above 15 (ref. 7). The resins obtained were named 2-resin, 5-resin, 10-resin, 20-resin, 30-resin, 40-resin and 100-resin. The number indicates the number of moles of furfural for 1 mole of acetylferrocene in the starting material. To examine the physical and chemical state of the iron by Mössbauer spectroscopy, 30- and 100-resins were prepared using ferrocene consisting of ⁵⁷Fe enriched to 62.5% (ref. 8). The resin was heated in a vacuum of $1 \times 10^{-3} \text{ mmHg}$ from room temperature up to 350°C for 4 h, then up to 400°C for 2 h. The heat-treated resin, still in its evacuated container, was

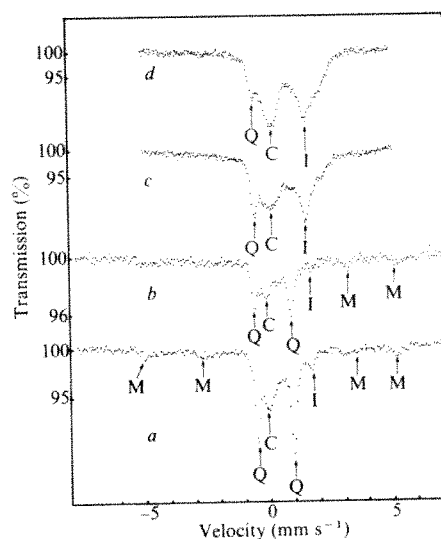
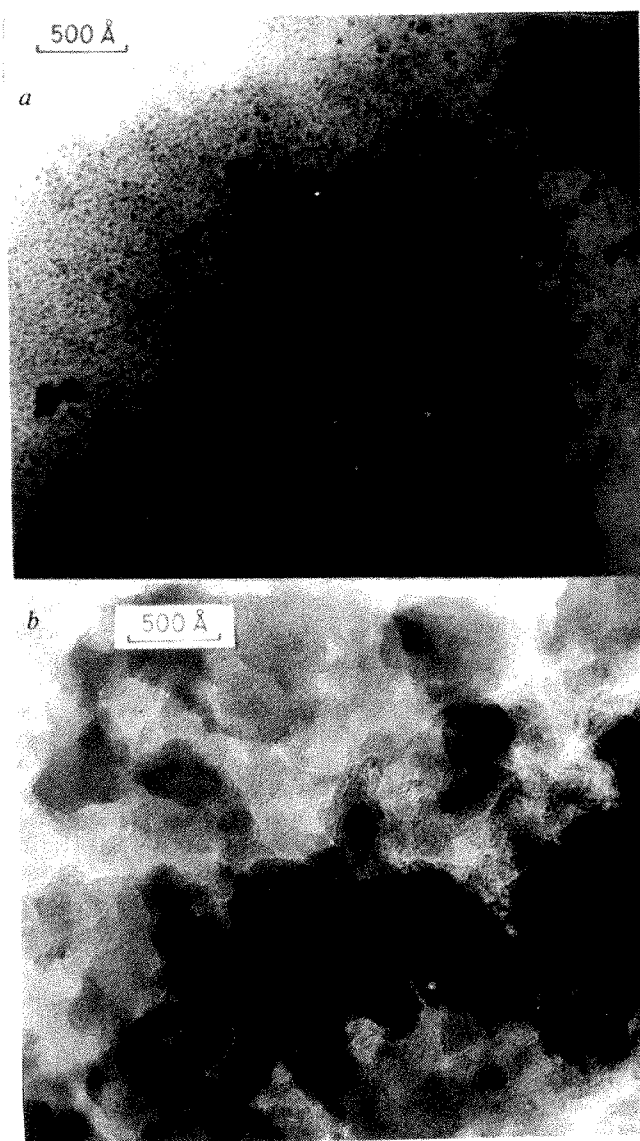
Table 1 Elemental analyses of resins heat-treated at 400 °C

Sample	Fe (wt%)	C (wt%)	H (wt%)	O (wt%)*
2-resin	13.20	69.07	3.68	14.05
5-resin	10.60	71.08	3.22	15.10
10-resin	7.96	72.01	3.58	16.45
20-resin	3.65	73.33	3.65	19.37
30-resin	2.54	75.06	3.77	18.65
40-resin	2.06	76.36	3.69	17.89
100-resin	1.00	77.14	3.78	18.08

* The percentage of oxygen is obtained by subtracting the percentages due to iron, carbon and hydrogen from 100.

placed in a glove box filled with argon; this was used for each measurement.

The resins heat-treated at 400 °C were susceptible to oxidation. In particular, 10-resin was pyrophoric on exposure to air. In the resin heat-treated at 400 °C, therefore, the iron liberated from the ferrocene skeleton would be present as ultra-fine particles or in a low valence state (i.e. Fe^+ or Fe^{2+}). As ferrocene is decomposed by heat-treatment above 400 °C, iron carbide is not formed but metallic iron is produced⁹. So there is probably no iron carbide in the resins heat-treated at 400 °C.

Fig. 1 Electron micrographs of the resins heat-treated at 400 °C, *a*, 2-resin; *b*, 100-resin.**Fig. 2** Mössbauer spectra of the resins heat-treated at 400 °C (4.2 K); *a*, 2-resin; *b*, 10-resin; *c*, 30-resin; *d*, 100-resin.

Infrared spectra showed that the resin structure was decomposed by heat-treatment at 400 °C (ref. 7). The X-ray diffraction patterns indicated that the matrix of heat-treated resin was a glass-like carbon. Results of elemental analysis of the heat-treated resin are shown in Table 1. Electron micrographs of 2- and 100-resins heat-treated at 400 °C are shown in Fig. 1. As shown in Fig. 1*a*, in 2-resin iron particles 10–25 Å in diameter were dispersed uniformly in the glass-like carbon, and larger iron particles (30–150 Å) were thinly scattered. This dispersion was also observed in 5- and 10-resins heat-treated at 400 °C. In 20- and 30-resins heat-treated at 400 °C, iron particles of irregular size (10–150 Å) were dispersed in the glass-like carbon matrix. However, in 40- and 100-resins heat-treated at 400 °C no iron particles were visible, as shown in Fig. 1*b*; the iron particles were evidently below 10 Å in size. In Table 2 are shown the X-ray diffraction data of 2-resin heat-treated at 400 °C (Table 2). The ultra-fine particles included iron of hexagonal close-packed (h.c.p.) ($a_0 = 3.30$, $c_0 = 5.21$) and distorted body-centred cubic (b.c.c.) ($a_0 = 2.95$) type structures. Iron of h.c.p. type structure has previously only been recognised at high pressures of 130 kbar and above¹⁰. These iron particles of h.c.p. and distorted b.c.c. type structures corresponded to those of 10–25 Å in Fig. 1*a*. Diffraction peaks due to the iron particles of 30–150 Å were not observable, since they were small in number. The X-ray diffraction patterns of 5- and 10-resins heat-treated at 400 °C were similar to that of 2-resin. No diffraction peaks due to iron particles were detectable in the other resins heat-treated at 400 °C. Mössbauer spectra of 2-, 10-, 30-, and 100-resins heat-treated at 400 °C are shown in Fig. 2. In the Mössbauer spectrum of 2-resin, four peaks marked M were due to magnetic splitting of ferromagnetic iron. The isomer shift of the two peaks marked Q was $+0.21 \pm 0.03 \text{ mm s}^{-1}$;

Table 2 X-ray diffraction data of ultra-fine iron particles in 2-resin heat treated at 400 °C.

Hexagonal close-packed iron		Distorted body-centred cubic iron	
$d(\text{Å})$	(hkl)	$d(\text{Å})$	(hkl)
2.85	(100)	2.09	(110)
2.61	(002)	1.48	(200)
2.51	(101)		
1.61	(110)		
1.48	(103)		
1.39	(112)		
1.30	(004)		
1.10	(203)		

the separation was $1.44 \pm 0.03 \text{ mm s}^{-1}$. These peaks were possibly due to the quadrupole splitting of h.c.p. iron. The peak marked I at $+1.70 \pm 0.03 \text{ mm s}^{-1}$ was attributed to Fe^+ , because the large isomer shift value agreed with that published for Fe^+ (ref. 11). The peak marked C, at $-0.08 \pm 0.05 \text{ mm s}^{-1}$, was due to iron cluster, because the isomer shift corresponded to that of iron cluster¹². In 10-resin, the peaks due to the magnetic splitting were weaker. In 30-resin, the peaks due to the quadrupole splitting were weaker, and the magnetic splittings were not detected. In 100-resin, only slight quadrupole splittings remained. That is to say, as the iron content in the resin decreases, the peaks due to the magnetic splitting and the quadrupole splitting successively weaken and disappear. The peaks due to iron cluster and Fe^+ are present. In the inorganic glass-like carbon prepared by heat-treatment of the acetyl-ferrocene-furfural resin, the liberated iron is in ultra-fine particles as shown in Fig. 1. The size of iron particles increases probably in the order of cluster, h.c.p. iron, distorted b.c.c. iron, and b.c.c. iron. In the resin heat-treated at 400°C , Fe^+ ion is stabilised. In the present study, however, it could not be shown how the Fe^+ ion is bonded chemically to the glass-like carbon matrix.

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Bomb ^{14}C and human radiation burden

RADIOACTIVE fallout from nuclear weapon tests has resulted in a significant increase in mean radiation dose received by the world's population. While fission nuclides such as ^{137}Cs and ^{90}Sr are important sources of internal radioactivity, notable characteristics of artificial ^{14}C production are (1) its long half-life ($5,730 \pm 40 \text{ yr}$), (2) easy access via the food chain to all key molecules of body tissue, and (3) relatively long residence times in both stratosphere and troposphere of four and eight years respectively² (cf. mean residence time of particulate nuclear debris in the troposphere $\sim 30 \text{ d}$ ^{3a}). Although ^{14}C emits a low-energy β -particle ($E_{\text{max}} = 156 \text{ keV}$), its ease of incorporation in genetic material and the associated implications have provoked concern among several eminent scientists over artificial production of this radionuclide, even prior to the major testing period⁴⁻⁷. Recent data on levels of bomb ^{14}C and residence times of carbon in the human body are now available⁸. Thus excess human radiation burdens from this radioisotope are evaluated in this article under the assumption that no radiation dose, however small, can be regarded as entirely harmless biologically. This assumption, albeit a major one, is still adopted for radiation hazard considerations in the absence of conclusive evidence otherwise.

The absorbed dose, D_N , to soft tissue/bone marrow and bone-lining cells from decay of natural ^{14}C is $0.69 \text{ mrad yr}^{-1}$ and $0.74 \text{ mrad yr}^{-1}$ respectively. Since ^{14}C concentrations during the nuclear era are normally quoted as percentage

excess, $\Delta(\%)$, over the natural level, corresponding ^{14}C doses are directly expressed as $D_N(1 + \Delta/100)$. Annual absorbed doses from ^{14}C , both natural and man-made, are estimated for 1953–1973 (see Fig. 1) using $\tau = 6 \text{ yr}$ and $\tau = 30 \text{ yr}$ as representative mean values of residence times of carbon in soft tissue and bone collagen respectively⁸. Plotted values apply to mature tissue but may be used with minimal error for tissues under growth, with the exception of bone collagen. For this latter component, an approximate dose is estimated using conditions of linear increase in collagen with no carbon turnover. In this situation, the annual dose for a 20-yr old is simply the averaged annual dose contributions received before this age and reaches a maximum of $1.07 \text{ mrad yr}^{-1}$ for bone-lining cells, delivered in 1984 to individuals born in 1965.

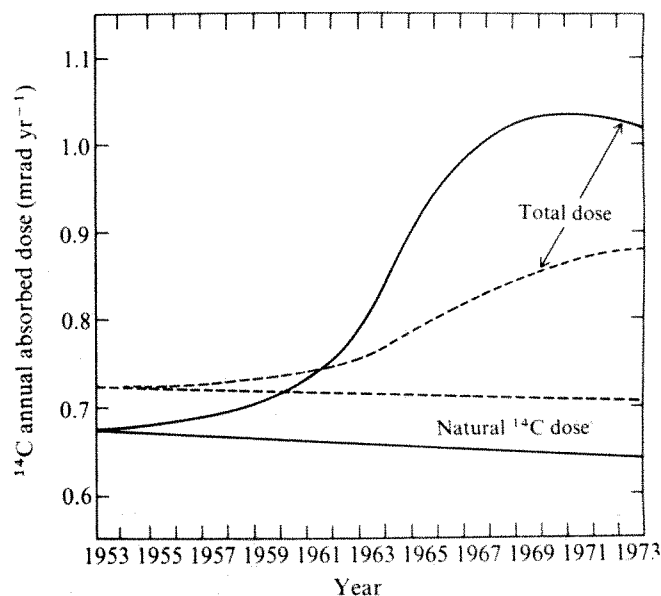


Fig. 1 Annual absorbed doses attributable to ^{14}C , 1953–1973. Total doses (natural + bomb ^{14}C) were calculated using tissue ^{14}C curves of Fig. 3, p.830; $\tau = 6 \text{ yr}$ and $\tau = 30 \text{ yr}$ were assigned as residence times to soft tissue/bone marrow and bone-lining cell carbon respectively. Natural ^{14}C dose rates were determined via theoretical consideration of the Suess effect¹⁰. ---, bone-lining cells; —, soft tissue/bone marrow.

Annual radiation doses for years 1975–2025 (see Fig. 2) are based on corresponding tissue ^{14}C levels. The latter data depend on future dietary ^{14}C content which has been estimated using (1) observed mathematical functions of atmospheric and oceanic ^{14}C levels⁹, and (2) a similar ocean water function but with an atmospheric ^{14}C activity decrease of 2% per yr, the present rate of decrease. The major uncertainty in Fig. 2 is associated with prediction of future atmospheric ^{14}C levels. With no further appreciable artificial production of this radioisotope, however, atmospheric ^{14}C content should reach pre-bomb levels by 2025, essentially via exchange of carbon with the oceans, and by dilution with inactive CO_2 (Suess effect). Again, natural ^{14}C data are included in Fig. 2, with estimates based on theoretical predictions of atmospheric $^{14}\text{CO}_2$ activities under the influence of the Suess effect alone¹⁰. Thus although environmental ^{14}C levels will approach pre-bomb concentrations by the first half of next century, net contributions from artificially produced ^{14}C are still appreciable.

For pertinent evaluation of possible biological effects of this radiation, total radiation received by any tissue over its critical years of function is more relevant than individual annual doses. Examples of cumulated doses to gonads (over 30 yr) and to bone marrow and bone-lining cells (over 60 yr)

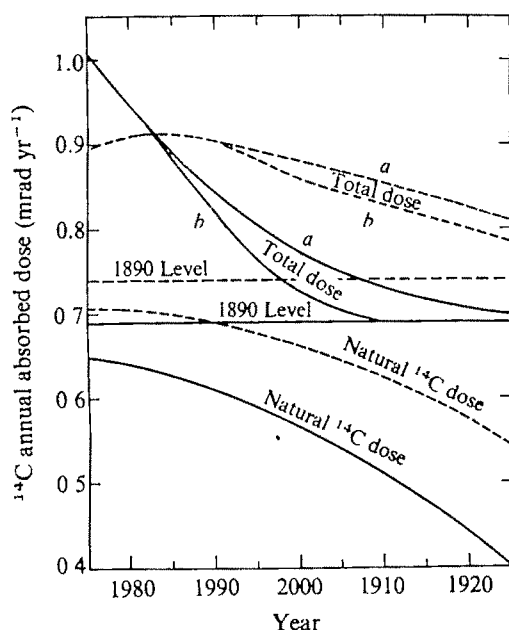


Fig. 2 Predicted annual absorbed doses attributable to ^{14}C , 1975–2025. Total doses to soft tissue/bone marrow and bone-lining cells were calculated from predicted tissue ^{14}C levels which are closely related to environmental ^{14}C activities. The latter data in line (a) were based on predicted future atmospheric and oceanic ^{14}C contents⁹, and in line (b) were based on an atmospheric decrease in ^{14}C content of 2% yr^{-1} . Natural ^{14}C dose rates were assessed as in Fig. 1. Linear extrapolation of upper curves (a) indicates that the total absorbed doses to soft tissue/bone marrow and bone-lining cells will return to natural levels by years 2035 and 2050 respectively. —, Bone-lining cells; —, soft tissue/bone marrow.

have been determined (see Table 1). Evaluation of the long-term genetic threat represented by any long-lived radioisotope utilises a quantity known as dose commitment, obtained *via* integration of dose over that time during which there is a significant contribution to the world population. For bomb ^{14}C , the dose commitment as calculated by UNSCEAR is 140 mrad and 170 mrad to soft tissue/bone marrow and bone-lining cells respectively³. These totals overestimate the potential radiation hazard from bomb ^{14}C , however, by total neglect of the Suess effect which, in the absence of artificial production of ^{14}C , would be expressed as a decrease in the tropospheric ^{14}C activity of ~40% below the natural level. Total accumulated doses in this article are obtained by addition of all annual absorbed doses in excess of that delivered by natural ^{14}C . Such a summation is performed until 2050 for bone-lining cells and to 2035 for soft tissue/bone marrow (see Fig. 2) to give values of 10 mrad and 8.5 mrad respectively. These totals, though well below ^{14}C dose commitments of the UNSCEAR report, are, we feel, more relevant for discussion of the real human radiation burden from bomb ^{14}C .

Table 1 Accumulated absorbed doses due to bomb ^{14}C

Period under consideration	Accumulated dose (mrad)	
	Soft tissue/bone marrow	Bone-lining cells
pre 1954	—	—
1955–1984	7.26	3.16
1964–1993	8.81*	4.95
1970–1999	8.31	5.69
1985–2014	6.65	6.57*
1965–2024	bone growth during initial 20 yr	24.80* (60-yr dose)

*Maximum doses.

Assessment of the human radiation burden from bomb ^{14}C is the aim of this paper; thus it is important to relate the quantities determined here, which belong obviously in the category of low radiation doses to cellular modification, particularly chromosomal DNA damage. Possible radiation-induced chemical changes which occur in DNA have been reviewed recently¹¹ while Table 2 indicates the probability of damage per rad to DNA and other genetic components of a cell. Information in column 3, applicable to bomb ^{14}C , should be regarded with caution since data in column 2 are compiled from numerous research projects performed under a diversity of experimental conditions—dose rate, type of irradiation, but notably, type of cell¹². On the other hand, results of recent experiments using ^{14}C -methyl thymidine incorporated in mammalian DNA, substantiate at least the quoted extent of single strand breakage¹³. Allowance is made in Table 2 for repair of single strand breaks (up to 85% in mammalian DNA¹⁴) especially since such repair has also been shown to result in normal replication¹⁵. Unfortunately, interpretation of probabilities of cell damage due to bomb ^{14}C remains difficult since the lesion(s) responsible for the visible expression of biological damage has yet to be identified. For example, if each chromosome break is a critical lesion, the total absorbed dose due to bomb ^{14}C implies an ultimate probability of damage to any cell of 2×10^{-3} to 10^{-4} . Thus, given a birth rate of 3% in a world population of 2.5×10^9 , 1,500–7,500 offspring would possess altered genetic material resulting in a wide range of consequences.

Table 2 Estimates of biological damage to reproductive cells from ^{14}C β -irradiation (30-yr accumulated dose)

Description of damage to genetic material	Extent of damage (probability per cell)	
	Typical values per rad of ionising radiation	Contribution from bomb ^{14}C β -irradiation*
Single-strand breaks	10	0.01†
Double-strand breaks	1	0.006
Chromatid breaks	0.002–0.24	1.2×10^{-6} – 1.4×10^{-3}
Chromosome breaks	0.002–0.01	1.2×10^{-6} – 0.6×10^{-4}
Damaged bases	5–15	0.03–0.09

*Calculations performed using maximum bomb ^{14}C 30-yr absorbed dose.

†Probability based on 85% repair of single-strand breaks.

Local effects from the $^{14}\text{C} \rightarrow ^{14}\text{N}$ transition involve possible consequences of nuclear recoil, excited electronic state, and modified valence state of the daughter nucleus. In each respect, exact location of a ^{14}C atom is expected to be critical; and in certain situations, the transmutation effect may be the dominant damage factor. On the other hand, distinction between consequences of β -irradiation and local events is difficult for such a low-energy β -particle. Consequently there is conflicting experimental evidence for and against a positive transmutation role of ^{14}C decay⁴. Long-term experiments involving a ^{14}C general labelling technique have been completed recently by Lee and co-workers¹⁷; although genetic damage could be attributed to β -irradiation effects alone, the possibility of a transmutation effect associated with specifically-labelled DNA was not dismissed. With this background of inconclusive experimental evidence, the approach of Totter *et al.*⁷ remains the best theoretical treatment of a transmutation role during ^{14}C decay. Two situations for bomb ^{14}C are considered, (1) transmutation=mutation, cf. Totter *et al.*, and (2) transmutation at only one type of carbon site in DNA=mutation. Resultant values (see Table 3) represent extreme cases of a potential transmutation effect; once again, visible expression of this damage to cellular components cannot be predicted.

Table 3 Theoretical assessment of the transmutation effect of ^{14}C (applied to bomb ^{14}C)

total dose to gonads from bomb ^{14}C $\approx$ 10 mrad	
time span involved = 80 yr	
thus mean annual dose to gonads (bomb ^{14}C) = 0.13 mrad	
cf. annual dose to gonads (natural ^{14}C) = 0.68 mrad	
dose rate due to bomb ^{14}C is $\approx$ 20% that due to natural ^{14}C	
disintegration rate of natural ^{14}C = 13.56 d.p.m. g^{-1} carbon	
wt of DNA per cell $\approx$ 8.6×10^{-13} g	
% of carbon in DNA $\approx$ 37%	
therefore no. of ^{14}C d.p.m. $\approx$ $13.56 \times 8.6 \times 10^{-13} \times 0.37$ per DNA per cell	
decays per DNA per cell per yr (natural ^{14}C) $\approx$ $13.56 \times 60 \times 24 \times 365 \times 8.6 \times 10^{-13} \times 0.37$	
$\approx$ 2.27×10^{-6} decays per cell per yr	
decays per DNA per cell per yr (bomb ^{14}C) $\approx$ $\frac{2.27 \times 10^{-6}}{5}$ decays per cell per yr	
$\approx$ 4.5×10^{-8} decays per cell per yr	
Case 1	
transmutation = mutation, thus probability of mutation	
$= 4.5 \times 10^{-8}$ per cell per yr	
birth rate $\approx$ 3%	
world population $\approx$ 2.5×10^9	
total no. of yr = 80	
total offspring affected $\approx$ 27,000	
Case 2	
transmutation at only one type of carbon site is responsible for a mutation, thus probability of mutation	
$= \frac{4.5 \times 10^{-8}}{40}$ per cell per yr*	
total offspring affected $\approx$ 700	

*Approximately 40 different carbon sites in DNA.

Owing to their obvious limitations, the preceding approaches can only represent crude attempts at assessment of genetic damage due to bomb ^{14}C . For more relevant consideration of the social implications of artificial production of this radionuclide, reference is made to the latest UNSCEAR report which contains risk estimates of radiation-induced genetic disorders in humans³⁰. These data are calculated with respect to damage to spermatogonia and oocytes, the germ cells most at risk, and are based on expected rates of damage at low doses and in conditions of chronic exposure, particularly applicable to ^{14}C . In the report, two approaches are employed to determine incidences of mutation of the zygotes—formed by union of male and female germ cells—per rad of low-dose irradiation administered to the parental generation. First, an incidence of damage of $\sim 1,500$ per 10^6 spermatogonia is obtained by extrapolation of genetic data involving mice to humans, taking into account the 20% increase in size of the human genome. The second value, 300 per 10^6 spermatogonia, is derived using a 3% spontaneous incidence in genetic damage among live born, together with a radiation doubling dose of 100 rads—that low-dose irradiation expected to double the natural mutation rate. Corresponding radiation damage to oocytes is considered negligible in comparison. Combination of these incidences of damage with 10 mrad, the total absorbed dose to the gonads due to bomb ^{14}C , yields upper and lower limits of 1,125 and 225 respectively for the number of individuals who will possess severe deleterious traits attributable to this radioisotope. It should be stressed that these figures have been derived using a linear dose-response relationship extrapolated to those levels of bomb ^{14}C , and should be assessed accordingly. Expression of this damage in first generation offspring is estimated at ~ 20 – $55/5$ –10 using an assumed range of dominance in man of 2%–5%³¹. Consideration of minor deleterious effects have not been included in this computation but are expected to be more abundant even if no visible damage is apparent. This fact explains to some extent the lower values obtained here compared with those calculated in the preceding analyses.

With respect to late somatic effects, in particular an increase in number of leukaemia and cancer/tumour victims from effects of ^{14}C decay, there is a similar lack of data obtained at low doses and under chronic exposure conditions. Again, a linear dose-response relationship is assumed for radiation burden assessment although there is

evidence of a sigmoid relationship for low-density ionising radiation¹⁸. Spiers¹⁹ uses a figure of 100 cases per 10^6 individuals per rad as the total risk of leukaemia and different types of bone tumours; this induction rate is derived from the UNSCEAR report³² and is rounded off owing to the many uncertainties involving dose-effect data. Thus this incidence of damage corresponds to a total of $\sim 2,000$ cases attributable to bomb ^{14}C ; again the role of transmutation of ^{14}C may be significant but this topic has been discussed recently by Tamers²⁰.

For both genetic and somatic considerations, the potential human radiation burden due to artificially produced ^{14}C and calculated using the assumptions stated is certainly significant and, therefore, cannot be dismissed. However, relative to the world population and in view of the time span over which this dose is delivered, some three to four generations, the conclusion of this study must be that such effects will occur unnoticed.

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Bomb ^{14}C as a biological tracer

THE release of ^{14}C in nuclear explosions, particularly those detonated in 1961–1962, has caused a major disturbance of the steady-state natural distribution of this radioisotope. The resultant pulse of bomb ^{14}C , detected initially in the atmosphere, has since been used extensively in evaluation of rates of exchange of carbon between major reservoirs of the dynamic cycle — notably atmospheric CO_2 and ocean water bicarbonate^{1–7}. Within the biosphere, however, investigation of human ^{14}C levels has been more limited despite the application of bomb ^{14}C as early as 1959 by Broecker and co-workers⁸ in the determination of residence times of carbon in several tissues. Perhaps the most specific result to emerge from initial research on this topic was the demonstration of almost total inertness of collagen in the adult human skeleton as evidenced by its failure to incorporate significant amounts of artificial ^{14}C (ref. 9). In other related ^{14}C measurements^{10–13}, a non-correspondence between contemporary human and atmospheric ^{14}C levels was often observed. Nydal and co-workers proposed a combination of three factors to account for such variations¹³ and concluded from a long term analysis of ^{14}C concentrations in human blood and hair samples that bomb ^{14}C enters the human body 1.4 years after production in the atmosphere, and remains there with a mean lifetime of 10 years. This article describes the continuation at Glasgow of an intensive investigation of the presence of bomb ^{14}C in humans, initiated by Harkness and Walton¹². Comparison by these workers of blood plasma protein ^{14}C data with predicted dietary ^{14}C levels¹⁴ has confirmed that a finite turnover of carbon in the human body does exist. Here, determination of ^{14}C activities of atmospheric CO_2 , dietary and human tissue samples enables a more detailed evaluation of residence times.

Atmospheric CO_2 was sampled monthly by adsorption in KOH solution (4 M) at three UK locations (see Fig. 1). Representative samples from each location were analysed for ^{14}C content since ^{14}C measurement of all samples was impracticable. Human tissues, normally about 20 g, were obtained unfixed from postmortem examinations. After washing, the tissues were homogenised and freeze-dried and subsequently samples were combusted directly to CO_2 or pretreated, before combustion, with Bloor's solvent (ethanol-diethyl ether, 3:1 v/v) to provide protein- and lipid-rich components for analysis.

Grossly diseased aortas from victims of atherosclerosis were forwarded from New Orleans, USA. An additional artery sample was obtained at Glasgow. Although the former samples had been stored in formalin, it was felt that this source of carbon contamination would be eliminated during lipid extraction. After washing, the intima was carefully stripped from the other two arterial layers; plaques were separated from relatively normal tissue and extracted overnight at room temperature with chloroform-methanol (2:1 v/v). The extract yielded on evaporation an oil from which lipids were selectively removed into ether-hexane (1:1 v/v). This solution was washed with warm saline solution, dried with Na_2SO_4 , and evaporated to dryness. A stream of nitrogen was passed over each residue for several hours to ensure total removal of solvent. Samples obtained in this way weighed typically 1–2 g.

Biological samples were combusted in a stream of oxygen and the resultant CO_2 absorbed in KOH solution. Details are given elsewhere¹⁵ of the subsequent purification of CO_2 necessary for gas-proportional anti coincidence counting. ^{14}C activities were measured in a 2.61 counter, filled to a pressure corresponding to one atmosphere at 15 °C; under these conditions the background count rate was 4.9 c.p.m. with an observed deviation of -0.01 c.p.m. per mbar rise in atmospheric pressure.

Tables 1 and 2 include the ^{14}C results of all human tissues analysed, after correction for isotopic fractionation by analysis of the $^{13}\text{C}/^{12}\text{C}$ ratio of each sample relative to the P.D.B. Chicago Limestone Standard. Corresponding theoretically

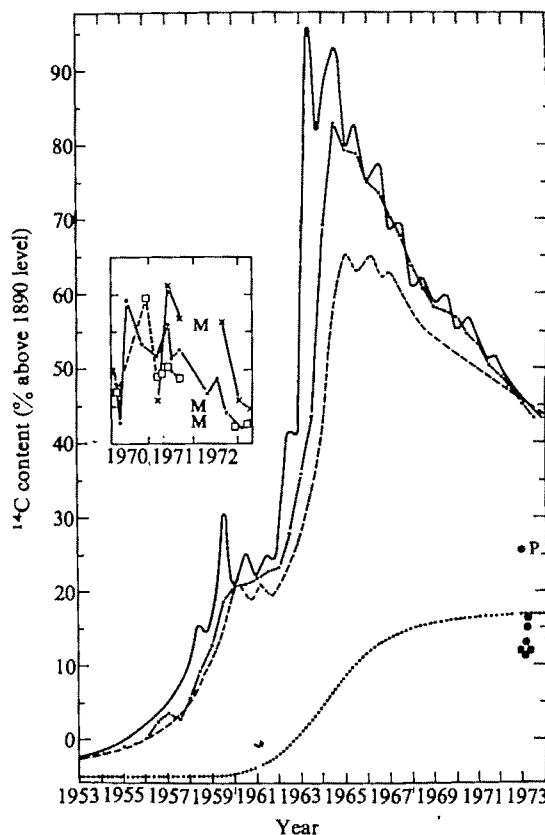


Fig. 1 Environmental ^{14}C concentrations, 1953–1973. ●, Fish samples obtained fresh in March 1973 (●P = plaice); M, meat obtained fresh in January 1972. —, N. Hemisphere troposphere; ---, S. Hemisphere troposphere., Mixed ocean layer; — · —, computed dietary level. Inset: $^{14}\text{CO}_2$ activities at UK latitudes, —●—, Lerwick (60°08'N, 01°11'W); —□—, Snowdon (53°03'N, 04°00'W); —×—, Chilton (51°31'N, 01°20'W).

determined values were evaluated by application of a suitable box-model treatment of carbon turnover in tissues. The potential of bomb ^{14}C in this system results from nature's unique labelling mechanism whereby ^{14}C incorporation in biological material yields a uniform distribution throughout each molecule—no particular molecular sites are favoured. ^{14}C , both natural and man-made, is assimilated by plants by photosynthesis with subsequent transfer to humans through the food chain. Thus the ^{14}C content of any tissue may be regarded as a function of dietary ^{14}C level and of the residence time of carbon in that tissue. From this basic model (Fig. 2a) it can be shown that, for adult tissue, in which an essentially stationary state exists (carbon input = output), the ^{14}C content at any time is given by the equation (see a similar expression by Jansen¹⁶)

$$\Delta_T^{nt} = \Delta_T^0 + \sum_{i=1}^n \left\{ \Delta_D^{ni} - \Delta_T^{(n-1)i} \right\} \left\{ 1 - \exp(-k_T i) \right\} \quad (1)$$

where T and D refer to tissue and diet respectively; $\tau = 1/k_T$, the mean residence/turnover time, is the average time required by a tissue for replacement of an amount of carbon equivalent to the tissue itself. Thus for a given τ value, and with i fixed at 0.5 yr, tissue ^{14}C concentration is dependent only on the cumulative differences between the previous ^{14}C activity and the mean ^{14}C content of the diet over the subsequent half-yearly interval. By appropriate choice of zero time (t_0 before the effects of nuclear weapon tests) a plot of Δ_T^{nt} against nt indicates build-up of bomb ^{14}C in any tissue.

Table 1 ^{14}C content of human tissues*

Donor/date of death†	Liver	Kidney	Spleen	Heart	Muscle	Lung	Brain	Miscellaneous
M/21: 9/71	54.6±0.9	51.2±1.6	46.0±0.8	42.4±1.4	55.9±1.3	53.9±1.6	—	Pancreas: 52.2±1.1 Testes: 56.0±1.3 Thyroid: 43.3±1.6
F/21: 9/71	55.2±1.0	53.1±1.2	54.8±1.6	54.5±1.3	51.7±1.3	51.1±1.1	52.3±1.1	Pancreas: 54.6±1.1 Ovaries: 50.6±2.0 Thyroid: 50.7±1.1 Testes: 49.8±1.8
M/37: 4/72	48.4±0.9	—	—	47.3±1.0	—	—	—	Thyroid: 37.9±1.5
M/39: 8/71	42.3±1.2	—	38.6±1.0	39.8±1.2	—	—	48.1±0.9	Pancreas: 46.0±1.2 Thyroid: 43.3±0.9 Testes: 37.8±1.0
M/42: 9/71	47.7±1.0	33.9±0.9	43.5±2.5	51.3±1.5	—	37.8±1.0	51.9±1.0	—
M/50: 1/71	43.3±0.8	44.6±0.8	—	52.3±1.9	50.2±1.3	—	—	—
M/61: 10/72	48.5±1.0 P45.0±1.1	45.3±0.8 P43.7±1.0	43.4±0.9 P46.2±1.0	50.4±0.8 P44.9±1.0	—	—	—	—
M/64: 11/72	47.5±0.8 P48.7±0.9 L47.2±1.1	46.3±0.9 P48.4±1.0	43.2±1.0 P42.8±0.9	— P40.9±0.9	—	44.0±1.2 P46.5±2.2	—	—
F/64: 4/71	—	49.5±1.0	45.6±1.2	41.5±1.5	48.2±1.0	—	49.1±0.9	Adipose tissue: 46.9±1.2 Ovaries: 47.5±1.5
M/67: 1/73	49.4±0.8 P49.4±1.0 L48.7±1.2	46.6±0.8 P48.5±0.9	—	46.8±0.8 P45.4±0.8 L51.1±0.8	—	—	—	—
M/72: 3/71	53.2±1.0	55.2±0.9	51.5±1.0	50.7±1.0	51.9±1.1	51.2±1.0	50.4±1.0	Pancreas: 54.7±0.9 Testes: 49.8±1.0 Bone(mf): 48.9±0.8 Bone(col): 10.4±2.7
F/87: 1/73	48.0±0.9 P49.1±1.0	46.2±0.9 P47.9±0.8	— P51.1±0.9	—	—	—	—	—
M/5: 9/71	52.8±0.9	50.4±1.5	44.9±0.8	—	52.5±1.0	—	50.5±0.9	Pancreas: 53.5±1.2

*These data are described in greater detail in refs 15 and 16.

†Age and sex of donor, month and year of death are given.

P, protein-rich; L, lipid-rich; mf, marrow fat; col, collagen; muscle analysed, forearm. See also footnotes to Table 2.

In practice, dietary ^{14}C content has not been measured with sufficient frequency over the last 20 yr but may be related to two more adequately monitored systems—atmospheric CO_2 and ocean carbonate/bicarbonate. Many such correlations are well established: plant life and cereals are good indicators of ambient atmospheric ^{14}C activities^{19,20}. In addition, the food types included in Fig. 1 reflect, fairly closely, environments of their origin, although recognition must be given to existence of carbon turnover in animals as well as in humans. Further relationships used in this study have been derived from previous environmental ^{14}C measurements²¹ coupled with data on stock/production of foodstuffs; the latter allows assessment of lag times between food growth and consumption (see Table 3).

With this knowledge, a comprehensive survey of dietary habits of the UK population was performed to evaluate

percentages of protein, lipid and carbohydrate supplied by different commodities. Note was made of the hemisphere of origin of food imports since marked disequilibrium between north and south hemisphere tropospheric CO_2 activities existed until 1971. The mean ^{14}C content of a typical UK diet

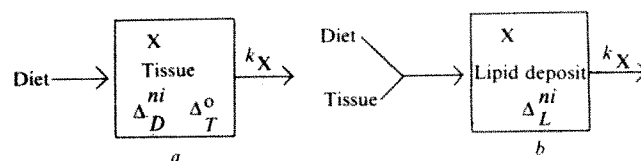


Fig. 2 Single compartment models of carbon turnover. X turns over with rate constant k_X , that fraction of X replaced in unit time (1 yr). X represents in (a), tissue carbon, supplied by a dietary source only; and in (b), arterial lipid carbon, supplied by the blood by material of dietary and endogenous origins. $\Delta_T^0 = ^{14}\text{C}$ content of tissue at t_0 , computed zero time; $\Delta_T^{ni} = ^{14}\text{C}$ content of tissue ni years after t_0 ; i is a suitable time increment—0.5 yr; $\Delta_D^{ni} = \text{mean } ^{14}\text{C}$ content of diet over the n^{th} i period after t_0 . For the non-stationary system: $N^{ni} = \text{no. of carbon atoms } ni \text{ yr after } t_0$ (tissue/lipid); $N_T^0 = \text{no. of carbon atoms at } t_0$ —at birth/11 yr of age.

at any time (see Fig. 1) is given by the expression

$$\Delta_D^{ni} = 0.24\Delta_{\text{TN}}^{ni} + 0.19\Delta_{\text{TN}}^{(n-1)i} + 0.16\Delta_{\text{TN}}^{(n-2)i} + 0.28\Delta_{\text{TN}}^J + 0.02\Delta_{\text{TS}}^{ni} + 0.035\Delta_{\text{TS}}^{(n-2)i} + 0.035\Delta_{\text{TS}}^J + 0.04\Delta_{\text{M}}^{ni}$$

TN and TS signify north and south hemisphere tropospheres respectively, while M denotes the mixed surface-ocean layer; $(n-1)i$ and $(n-2)i$ denote lag-time effects and J refers to the preceding period of maximum photosynthesis.

For tissues under growth, non-stationary conditions necessitate a modified approach. Use is made here of the general shape of growth curve obtained for most tissues²²; this curve, to a first approximation, may be regarded as a combination

Table 2 ^{14}C content of human tissue

Donor	Sample	$\Delta(\%) \pm 1\sigma$
M/66: 3/73	Liver (normal)	50.0±0.9
	Liver (cancered)	55.2±1.4
M/63: 2/73	Spleen (normal)	51.0±0.9
	Liver (cancered)	53.0±1.2
M/59: 2/73	Kidney (cancered)	48.1±0.8
M/68: 1963	Intimal lipid	4.8±0.6
M/67: 1964	Intimal lipid	9.7±0.9
M/69: 1964	Intimal lipid	17.9±0.8
F/86: 6/73	Intimal lipid	47.1±0.9
	Adventitia	31.6±0.8

*Normally, sample activities are quoted initially as $\delta^{14}\text{C}$, the per mil enrichment over $0.95 \times \text{NBS Oxalic Acid Standard activity}$ (=1890 wood). Here, after correction for isotopic fractionation using the Lamont VIII formula¹⁷, final values, expressed as $\Delta(\%)$, are shown in column 3. ^{14}C concentrations defined on this scale can be regarded as the percent deviation from pre-bomb or natural levels; $\Delta = 0\%$ corresponds to a natural ^{14}C concentration, and $\Delta = 50\%$ to a concentration $1.5 \times$ natural level. Errors, arising from uncertainties in ^{14}C activity measurement only, are quoted to one standard deviation ($\pm 1\sigma$).

Table 3 Dietary information

Main food categories	Contribution to typical diet (%)	Environmental relationship, Δ_D^{ni}
Milk/dairy products	23.0	Δ_T^{ni}
Cereals/flour/sugar	31.5	Δ_T^{ni}
Meat/poultry	34.0	$\Delta_T^{(n-1)i}, \Delta_T^{(n-2)i}$
Fruit/vegetables—fresh	3.4	Δ_T^{ni}
—other	3.7	$\Delta_T^{(n-1)i}$
Fish	4.4	Δ_M^{ni}

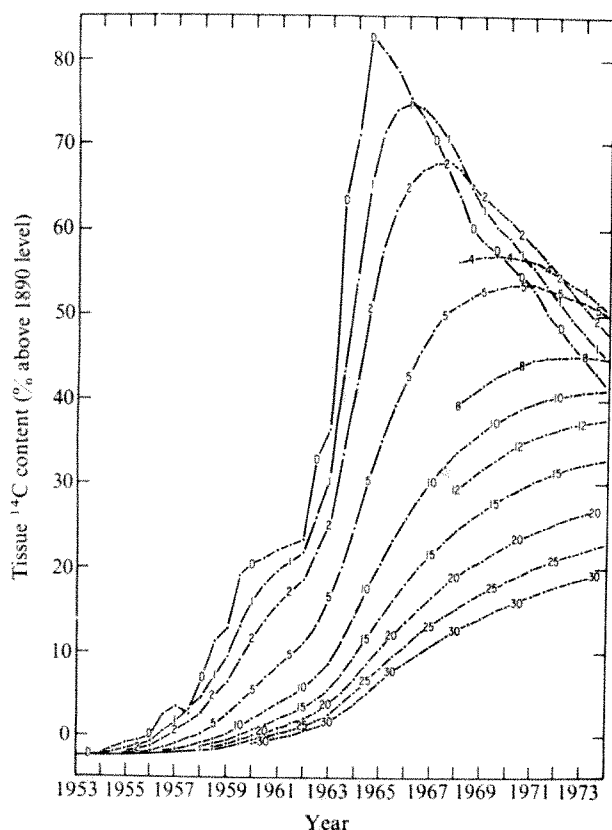
of two linear growth periods: from birth to 11 yr, and from 11 to 20 yr. In these conditions the comparable equation is

$$\Delta_T^{ni} = \Delta_T^0 + \sum_{n=1}^i \left\{ 1 - \frac{N_T^{0+}(n-1)(N_T^i - N_T^0)}{N_T^{ni}} \exp(-k_T i) \right\} \times \left\{ \Delta_D^{ni} - \Delta_T^{(n-1)i} \right\} \quad (2)$$

Since the first 2 yr after birth are best expressed by an exponential growth function, N_T^0 , rather than the absolute number of carbon atoms at birth, is that value obtained by extrapolation to birth (t_0) of the linear section of growth. Both N_T^0 and $N_T^i - N_T^0$, the increment in number of carbon atoms per 'i' time period, may be expressed as fractions of the mature tissue. For the 5-yr-old tissue series, errors involved in basic assumptions of the non-stationary system are considered too great to justify such a treatment. Nevertheless, ^{14}C activities of this series, with the exception of one organ, reflect dietary ^{14}C levels of the 18-month period prior to analysis. The different dietary pattern of a child may be responsible for the observed low ^{14}C content of spleen.

Model treatment of lipid accumulation in arteries also

Fig. 3 Predicted ^{14}C content of mature tissues, 1953–1973. Each curve represents a different mean residence time of tissue carbon. Steady-state conditions and first-order kinetics of carbon turnover are assumed to exist. —●—; $\tau = i$ yr; —D—; $\tau = 0$.



involves a non-stationary system. Numerous pathological findings indicate a linear incidence in number of atherosclerotic lesions with time from 20–25 years of age²³. Thus on the basis of a corresponding increase in amount of carbon deposited, the corresponding version of equation (2) is:

$$\Delta_L^{ni} = \Delta_L^{(n-1)i} + \sum_{n=2}^i \left\{ 1 - \frac{(n-1)}{n} \exp(-k_L i) \right\} \times \left\{ \Delta_L^{ni} - \Delta_S^{(n-1)i} \right\} \quad (3)$$

where L and S refer to lipid and source carbon respectively the latter is taken to be of composition similar to that of

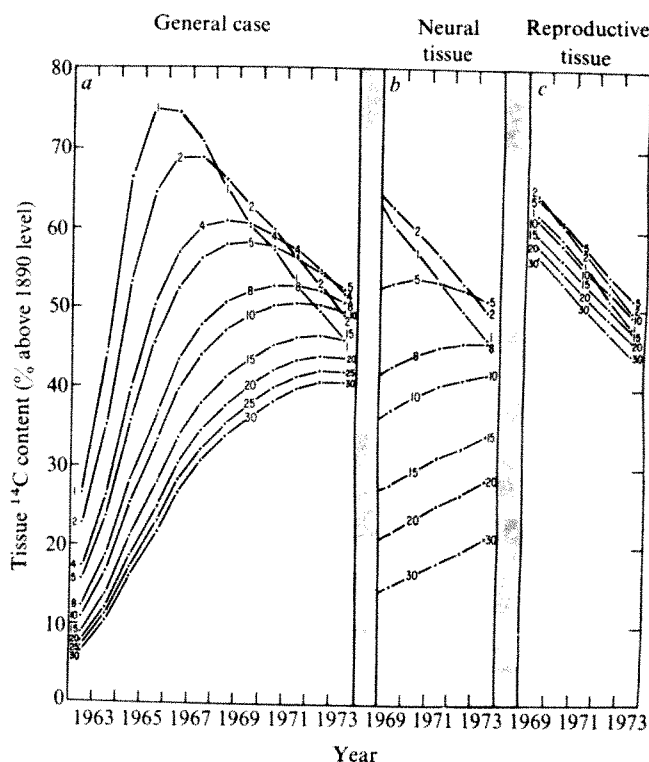


Fig. 4 Predicted ^{14}C content of tissues under growth, 1953–1973. Non steady-state conditions exist: (a) The general series of curves was based on two linear growth periods—birth–11 yr, and 11–20 yr. (b) For brain tissue, curves were computed for linear growth from birth–5 yr; thereafter steady-state conditions were applied. (c) For reproductive tissue, equation (1) was applied from birth to 15 yr, followed by linear growth from 15–20 yr. —●—; $\tau = i$ yr.

corresponding lipids in blood—30% dietary and 70% endogenous origins²⁴. Although most tissues are capable of preparation of cholesterol and its esters, two major components of the arterial extract, liver and gastrointestinal tract are considered to be main sites for biosynthesis of these fractions²⁵. Thus each Δ_S term is subdivided as follows:

$$\Delta_S^{ni} = 0.3\Delta_{D_2}^{ni} + 0.7\Delta_T^{ni}$$

Δ_T^{ni} data were obtained from estimated ^{14}C activities of a relevant tissue such as liver, while dietary emphasis was placed on those constituents high in cholesterol, total fat, and saturated fatty acid content; in this case

$$\Delta_{D_2}^{ni} = 0.48\Delta_{TN}^{ni} + 0.09\Delta_{TN}^{(n-1)i} + 0.28\Delta_{TN}^{(n-2)i} + 0.095\Delta_{TS}^{ni} + 0.035\Delta_{TS}^{(n-2)i} + 0.02\Delta_M^{ni} \quad (4)$$

Figures 3–5 contain series of curves computed from Δ_D^{ni} input relevant to each model, each curve corresponding to a

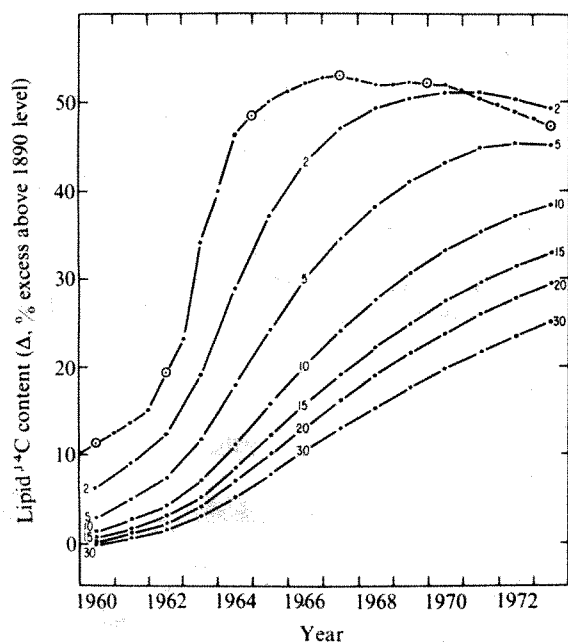


Fig. 5 Predicted ^{14}C content of arterial lipid deposits, 1953–1973. A similar situation to Fig. 4 exists, but with a different source of carbon input (30% dietary, 70% endogenous). No deposit was present at zero time, t_0 . —●—: $\tau = i$ yr; —○—: $\tau = 0$.

different mean residence time of tissue carbon. Comparison of the contents of Table 1 with the relevant figure yielded estimates of the turnover time, τ years, of carbon in that tissue. Uncertainties in this comparison are related to (1) ^{14}C activity measurement ($\pm 1\sigma$) and (2) diet-atmosphere relationship (estimated at ± 1 yr). Figure 3 shows that, for τ values 0–5, uncertainty in the diet-atmosphere relationships is predominant, while for longer residence times, both (1) and (2) are equally significant. Thus sensitivity of the bomb ^{14}C tracer method is expressed in terms of years rather than months. At this level of resolution, differences in turnover rates of normal against cancerous tissue are undetectable, certainly at the time of sampling. Similarly, for the purposes of this study, protein, lipid and whole tissue carbon exhibit no significant τ value variation. On the other hand, the long residence time obtained for bone collagen confirms previous findings^{9,14} of comparative inertness of skeletal carbon relative to soft tissue.

Significant variations in τ values exist, not only in tissues of

one donor, but also between the same tissues in different individuals, with no apparent trends as a function of age. Of the soft tissues, only thyroid carbon can be assigned, with confidence, a residence time ≥ 9 yr; elsewhere, in many cases, choices of τ values/range of τ values exist. This ambiguity does not imply that both selections are possible but merely signifies the inability of the model to distinguish between certain residence times owing to the proximity, at the time of analysis, of certain curves. However, it is difficult to reconcile the smaller of two choices of τ with the number of tissues ($> 25\%$) in which a residence time ≥ 8 yr is indicated. Comparison of Figs 3 and 4 shows that, for $\tau = 0/1$, there is negligible difference between corresponding Δ_T curves; yet about 35% of both 21-yr-old tissue series have ^{14}C content higher than either corresponding data from contemporary donors, or Δ_D values at the period of sampling. Considered together, these tissue results imply a residence time > 0 , but typically 2–5 yr. Thus as shown in Table 4, preference is given to upper τ ranges. In view of limitations imposed on the model at this time, a representative value of the mean turnover time of soft tissue carbon is taken as 6 ± 4 yr. This figure must be regarded as a maximum estimate which includes recycling of carbon within a tissue as well as interaction between different organs. Obviously the advantage of the bomb ^{14}C approach lies in detection of slow turnovers, typically > 6 yr; results can then be used to complement short term tracer studies which often fail to establish accurate upper limits on turnover rates. It should be stressed that τ values determined here are representative of each tissue in its entirety, or at least of its protein-lipid fractions. Extreme variations in metabolic rates must exist within tissues then, if results of this study are considered in conjunction with previous experimental observations which indicate much faster turnover rates of tissue amino acids^{26,27}.

Further application of bomb ^{14}C as a biological tracer is illustrated by Table 5 which implies a residence time, for carbon in intimal lipid deposits, of at least 5 yr. In the context of the model used, however, $\tau > 10$ yr seems more probable, provided that lipid is deposited directly from the blood. Another possible mechanism of lipid accumulation—*in situ* production—has been proposed²⁸, particularly for phospholipid build-up; accordingly, low Δ_L values may reflect ^{14}C activities of the endogenous precursor(s) of deposited carbon. In this respect, the ^{14}C content of adventitia (outer arterial layer) indicates the presence of about 25% natural ^{14}C , probably attributable to collagen²⁹. These data, while insufficient for detailed interpretation, encourage further measurements along a similar theme to determine the possible pathogenesis or timescale of treatment of atherosclerosis.

Table 4 Most probable residence times (τ , yr) of tissue carbon

Donor	Liver	Kidney	Spleen	Heart	Muscle	Lung	Brain	Testes/ Ovaries	Pancreas	Thyroid
M/21	1–6	8–10	15	20–30	1–6	5–8	—	1–10	7–9	15–25
F/21	1–6	6–8	1–7	1–7	8–10	8–10	5–6	10–15	1–7	9–10
M/37	7	—	—	7	—	—	—	5–6	—	—
M/39	9	—	11	10–11	—	—	7	—	7–8	11–12
M/42	7	13–14	8–10	5–6	—	11–12	5–6	—	—	9
M/50	8–9	8	—	5–6	6	—	—	10–12	—	—
M/61	6–7	8	9	6	—	—	—	—	—	—
M/64	P 8	P 9	P 7–8	P 8–9	—	—	—	—	—	—
	7	7–8	9	—	—	8–9	—	—	—	—
	P 6–7	P 6–7	P 9–10	P 10–11	—	P 7–8	—	—	—	—
F/64	L 6–7	—	—	—	—	—	—	—	—	—
	—	6–7	8	9–10	7	—	6–7	7	—	—
	6–7	7–8	—	7–8	—	—	—	—	—	—
M/67	P 6–7	P 6–7	—	P 8	—	—	—	—	—	—
M/72	L 6–7	—	—	L 5–6	—	—	—	—	—	—
	5	1–4	6	6	5–6	6	6	6	4–5	—
	7	7–8	—	—	—	—	—	—	—	—
F/87	P 6–7	P 7	P 5–6	—	—	—	—	—	—	—

M/72 Bone collagen, $\tau \geq 30$ yr; marrow fat, $\tau = 6$.

M/63 Spleen (normal), $\tau = 5–6$; Liver (cancerous), $\tau = 2–4$.

M/66 Liver (normal), $\tau = 5–6$; cancerous = 3–4.

M/59 Kidney (cancerous), $\tau = 6–7$.

Table 5 Mean residence times (τ) of arterial carbon

Donor	Sample	τ (yr)
M/68	Lipid extract	10-30
M/67	Lipid extract	5-25
M/69	Lipid extract	2-10
F/86	Lipid extract	0-5
	adventitia	15

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Lunar cycle and timing of mating season in Serengeti wildebeest

WILDEBEEST (*Connochaetes taurinus* Thomas) herds numbering nearly 1 million (refs 1, 2) on the Serengeti Plains, Tanzania, produce about 80% of their young during a 3-week period in January or February³. This is short compared with the 2-month birth period of the wildebeest in South Africa⁴, or to the 3-4-month birth period of buffalo and other grazers in the Serengeti⁵. A suggested advantage for the closely synchronised wildebeest birth period is that it reduces the probability of hyaena (*Crocuta crocuta* Erxleben) predation on the young^{3,6-8}. I report here evidence which suggests that the lunar cycle is the external timer for the synchronisation.

I have calculated individual conception dates from records of (1) foetus weights collected randomly and (2)

birth dates. Conception date can be calculated from foetus weight (ref. 9) (Fig. 1), and from birth date with a known gestation length. This lies between 8 and 9 months (refs 10, 11) and I have assumed 255 d.

Figure 1 shows the frequency distribution of conception dates from a random sample of 33 foetuses collected in 1971. Also shown are conception dates for 1964 and 1965, and the mating period for 1962 and 1963, all of which were calculated from information given by Watson¹¹. The distribution of conception dates for 1964, 1965 and 1971, together with other evidence³, show that conceptions are synchronised into one discrete time period with a relatively sharp onset. I have called this the 'mating period'.

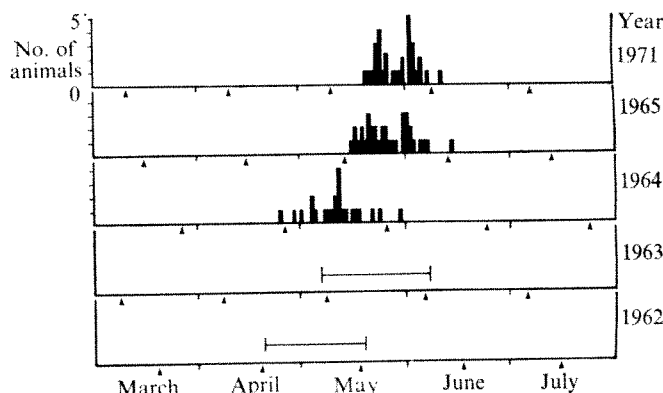


Fig. 1 Distribution of wildebeest conception dates in different years. Full moon is indicated by arrows. Conception dates were obtained from the relation between foetus age in days (t) and its weight in grams (W): $W^{1/3} = a(t - t_0)$, where t_0 is 20% of gestation length (t_g) and equals 51 d. 'a' is a growth constant and equals $W_g^{1/3}(t_g - t_0) = 0.143$. W_g is the birth weight of 25 kg. For the few small foetuses under 100 g, age was calculated directly from growth curves extrapolated from cattle¹³.

In Fig. 1 the times of full moon are shown by arrows. The mating period, where adequately observed (1962-1965, 1971), falls between the full moons. It starts approximately at the full moon and covers the new moon period. If there was no association between the timing of the mating period and the occurrence of full moon, the latter would be expected in the middle of this mating period on some occasions. To test for such an association I compared the starting date of the mating period with the date of the nearest full moon (Table 1), for there is additional information on the starting date derived from calving in the years 1968 and 1972 in a population 50 km to the east in Ngorongoro Crater, and also in the Serengeti in 1973 (A. and J. Root, personal communication). Estes⁹ observed the calving in three discrete herds in Ngorongoro in 1973.

If there was no relation between starting date and full moon then these dates should fall randomly within 15 d

Table 1 Absolute difference between full moon and start of the mating period

Place	Year	Start of mating period	Full moon	Difference between start and full moon (d)
Serengeti	1962	20 April	19 April	1
Serengeti	1963	7 May	8 May	1
Serengeti	1964	25 April	26 April	1
Serengeti	1965	16 May	14 May	2
Ngorongoro	1967	21 May	23 May	2
Ngorongoro	1971	15 May	10 May	5
Serengeti	1971	20 May	10 May	10
Ngorongoro a	1972	25-27 April	28 April	3
b	1972	25-27 April	28 April	3
c	1972	25-27 April	28 April	3
Serengeti	1972	27 April	28 April	1

either side of any full moon. Table 1 shows that 10 of the 11 dates fall within 7 d and 9 fall within 5 d of the full moon, strongly suggesting a relationship (the latter figures have $\chi^2=7.25$ with Yates correction, d.f.=1, $P<0.01$).

Supporting evidence for this relationship comes from the dates when first calves were observed by the park warden M. Turner¹² in the years 1956–1965. Conception dates have been calculated from these (Table 2). If there was no relationship with the moon, then these dates should fall at any time during the 30 d since the preceding full moon. In fact in eight out of ten years the first calculated conception fell within the first 10 d following the full moon, and all fell within the first 15 d ($P<0.01$, Kolmogorov-Smirnov test¹³). Wildebeest can delay birth for a short time⁸ but not to the extent that it could have produced the above observations.

Table 2 Conception dates calculated from first observed calving dates

First observed calving date	Conception date	Time since full moon (d)
11 November 1956	29 February 1956	4
4 December 1957	25 March 1957	10
16 December 1958	5 April 1958	2
6 December 1959	27 March 1959	3
2 December 1960	23 March 1960	11
20 December 1961	9 April 1961	9
12 November 1962	1 March 1962	9
27 October 1963	13 February 1963	5
23 November 1964	12 March 1964	14
28 December 1965	17 April 1965	2

All conception dates have been calculated using the mean gestation length of 255 d. This may be 5–10 d shorter or longer. Consequently until gestation time is more accurately known, one cannot say which lunar phase is important, but only that some aspect of it, as yet undetermined, is influencing conception.

Figure 1 shows that conceptions take place at approximately the same time each year. For conceptions to occur only in the lunar cycles of April and May an external variable showing an annual rhythm must also be influencing the animals. Although at this latitude (3°S) day-length alters by only 20 min through the year and is changing at its slowest in May (Fig. 2), it is possible that a combination of photoperiod and lunar cycle may act to trigger mating.

Alternatively local climatic conditions could act as a trigger for they are changing markedly at the start of mating in April (Fig. 2). In particular, rainfall and the amount of green grass upon which the animals feed¹⁴ decline rapidly at this time. This hypothesis is illustrated in Fig. 3. When rainfall or some related factor such as

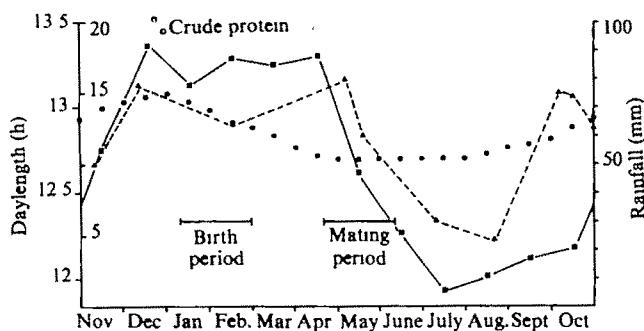


Fig. 2 The birth and mating season of Serengeti wildebeest in relation to daylength (●), mean monthly rainfall 1963–1972 (■), and % crude protein in the diet in 1971 (▲).

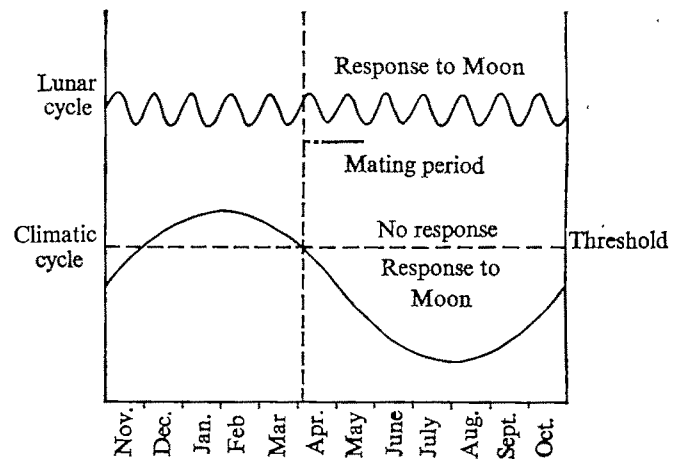


Fig. 3 Suggested model for the synchronisation of conceptions in wildebeest. When the climatic cycle (rain, food) falls below a threshold the animals become sensitive to the lunar cycle which switches on ovulation or allows conception to occur.

nutritional intake falls below a certain threshold, then the animals may become sensitive to changes in the lunar cycle. This still leaves a choice of two or three cycles to choose from. It is possible that if all females conceive in the first lunar cycle after the beginning of the decline in rainfall, then no conceptions would be observed in the following potentially suitable cycles. Indeed the birth data of Estes¹ in Ngorongoro show that some but not all small herds mate over a 2-month period, the distribution of conceptions being bimodal, with each peak coincident with each new moon. The data indicate that if the full moon occurs in the first half of April then conceptions are delayed by one more lunar cycle. These hypotheses can be tested by making predictions on the timing of the mating and birth seasons for future years.

To my knowledge this is the first case of a strong relationship between conception and the lunar cycle in mammals. There may be a similar effect in Australian water-buffalo (*Bubalus bubalis*)¹⁴ and perhaps a small effect on man¹⁵. For other mammals the data remain inconclusive¹⁶. The effect of lunar cycles may still be of relevance to the timing of breeding seasons in tropical regions, however.

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Passage of *Trypanosoma brucei rhodesiense* through the peritrophic membrane of *Glossina morsitans morsitans*

THE developmental cycle of African sleeping sickness trypanosomes in tsetse flies (*Glossina* spp.) as described by Robertson¹ has recently been questioned², especially the route whereby trypanosomes get from within the mid-gut of the fly to the salivary glands, where they develop into forms capable of infecting fresh mammalian hosts. We have shown² that trypanosomes developing in the mid-gut of *Glossina morsitans morsitans*, which are usually regarded as possessing low penetrative powers, are able to penetrate and pass through the gut cells of the fly. We present here more evidence of the penetrative powers of the fly mid-gut forms of *Trypanosoma brucei rhodesiense*—penetration of the peritrophic membrane of *G. m. morsitans* before their penetration of the gut cells.

An essential part of the developmental cycle of *T. brucei* spp. trypanosomes in the fly is the passage of the trypanosomes from the endoperitrophic space of the midgut to the ectoperitrophic space. The traditionally accepted route for this passage¹ is for the trypanosomes to escape from the confines of the peritrophic membrane around its often ragged posterior ends, where the mid- and hindgut join, and so gain access to the ectoperitrophic space. It has been suggested that trypanosomes *en route* to the salivary glands have to re-enter the endoperitrophic space by penetrating

the peritrophic membrane in the region of the proventriculus. Here the membrane is freshly secreted and hence soft^{3,4}, and electron micrographs of trypanosomes penetrating the peritrophic membrane in this region have been published⁵. This portion of the peritrophic membrane has also been suggested as a possible site for the initial access of the trypanosomes to the ectoperitrophic space^{6,7}. The penetration we describe, however, takes place in the middle section of the mid-gut, halfway between the proventriculus and the junction of the mid- and hindgut.

The techniques for the preparation of infected blood meals, the feeding, details of infecting trypanosomes and dissection of the flies, together with those for fixation and EM examination, were as used previously². Throughout our studies of this model, flies not sacrificed continued to harbour trypanosomes, and a satisfactory proportion developed mature salivary-gland infections and transmitted the infection to new mammalian hosts.

We found that as digestion of the infected blood meal proceeds and the volume of the material within the peritrophic sac diminishes, the peritrophic membrane becomes thrown into folds, and pockets are formed which trap the trypanosomes (Fig. 1a). The peritrophic membrane probably has several layers, the most easily recognisable of these being an inner osmophilic layer and an outer, paler, wider and more diffuse one. These layers separate (Fig. 1b) and the trypanosomes penetrate the osmophilic layer and gain entry to the space between the separated layers of the membrane (Fig. 1c). Penetration takes place most commonly at the apex of the membrane folds, which is the site

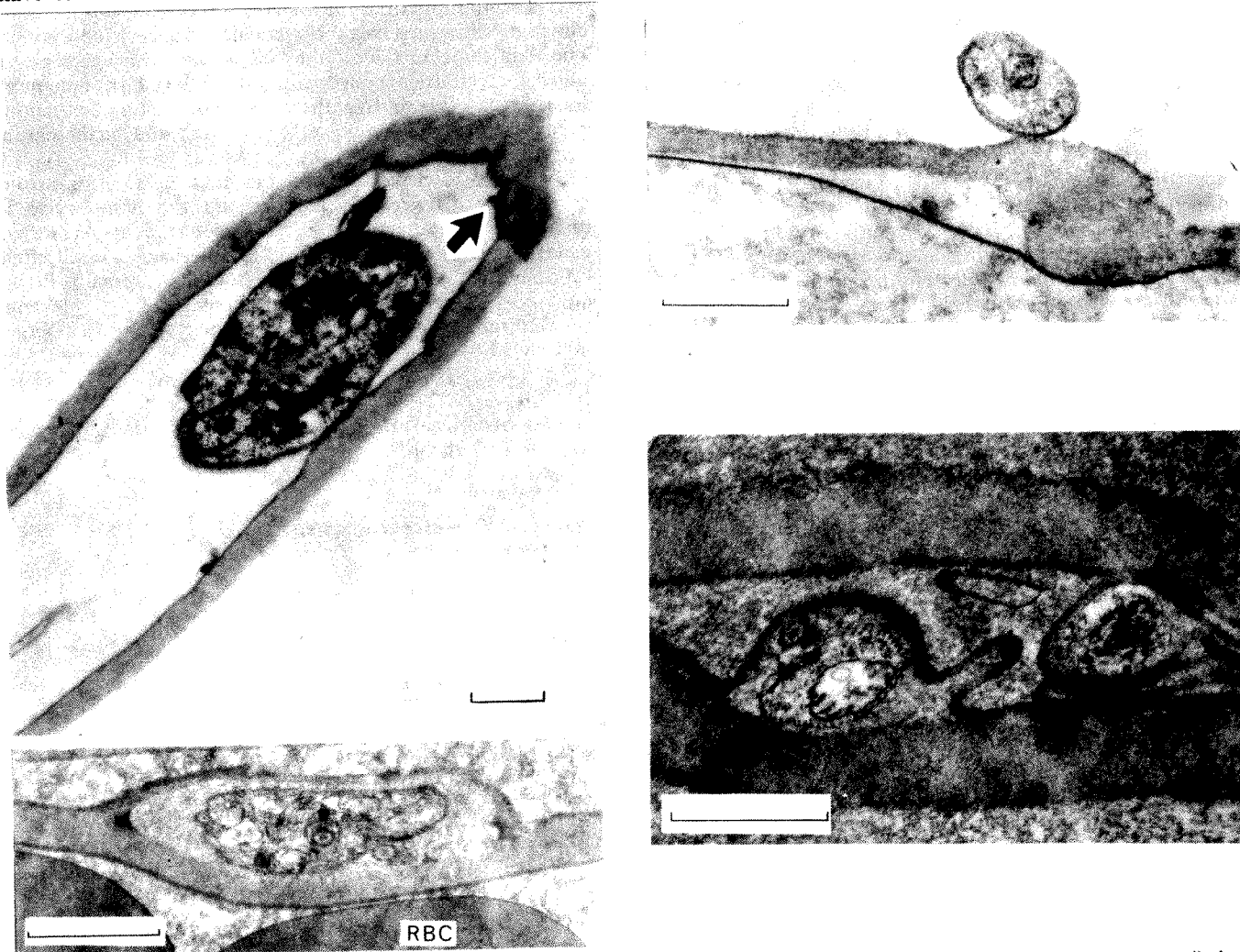


Fig. 1a A trypanosome trapped in a fold of the peritrophic membrane; note the 'buckling' of the inner layer at the apex (arrowed). b, Separation of the peritrophic membrane layers. c, Two trypanosomes lying between layers of the membrane in a fold. d, A trypanosome lying within the outer thicker layer. The cells of the blood meal (RBC) are below the peritrophic membrane. Bars represent 1 μ m.

where the separation of the layers is most frequently seen, particularly where the osmophilic layer is on the inside of the curve. Figure 1d shows trypanosomes lying within the thick layer of the peritrophic membrane.

Penetration of the peritrophic membrane occurred throughout the central two-thirds of the midgut, and its frequency was related to the trypanosome concentration, which was greatest in these central sections. We found no evidence of penetration occurring in the hindmost portion of the midgut, and the few trypanosomes found at this level appeared moribund. Nor did we find trypanosomes involved in the peritrophic membrane anterior to this midgut region. If any trypanosomes initially entered the ectoperitrophic space through the soft membrane in the proventricular region⁵ they had disappeared within 70 h of the infected feed, and we found no evidence of any trypanosomes outside the membrane until 10 d had elapsed.

Nine days after an infected feed all the trypanosomes were still within the peritrophic sac; by 11 days most of the trypanosomes had passed to the ectoperitrophic space, only 5% remaining within the sac, and a similar proportion were still involved in active penetration. The passage of successive waves of trypanosomes across the membrane from additional infected feeds took less time to complete—12 d compared with 14 d for a single infected feed.

The peritrophic membrane was found to be separated into two layers only in the presence of trypanosomes. Digestive enzymes may have a role in this process, but difference in rigidity between the layers of the membrane could certainly account for the separation seen at the sharp folds which occur when the sac contents decrease during the digestion of a blood meal. Lehan⁶ has shown in *Stomoxys calcitrans* that the thin layer of the peritrophic membrane contains no chitin (a rigid material) while the underlying layer does.

Epidemiological studies of the vectors of African sleeping sickness have traditionally relied on examination of the tsetse fly salivary glands for evidence of infection. In areas of endemic disease, the infection rates recorded by this method often fail to reach 0.8% (ref. 9). Any alternative life-cycle pathways of trypanosomes within the vector might suggest additional sites to examine within the fly when looking in the field for potential transmitters of African trypanosomiasis. Are we at present overlooking a large number of tsetse flies already capable of transmitting disease by only examining the salivary glands? Are the salivary glands continuously primed with trypanosomes coming from other parts of the fly (the haemocoel for instance) so as to maintain the population of metacyclic trypanosomes; or, once primed, is this sufficient to maintain salivary gland infection for the rest of the life of the fly? These are fundamental questions which a detailed study of all stages of the life-cycle of the trypanosomes in the fly would resolve.

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Rhodopsin content and electroretinographic sensitivity in light-damaged rat retina

EXPOSURE of rats to continuous illumination leads to cellular deterioration within the retina and a concurrent reduction of electroretinogram (ERG) responses^{1,2}. Grignolo *et al.*³ showed that at early stages, this 'damage' due to light is hypertrophic in that tubules and vesiculae appear in receptor cell outer segments. They surmised that losses in retinal functioning are initially due to disorganisation rather than degeneration. But, experiments on recovery from light damage⁴ indicate that ERG sensitivity returns as the rhodopsin-containing disks of the outer segments are regenerated even though their original lamellar structure is not restored. It is thereby implied that as long as visual pigment is present at functional sites, the normal structure of the outer segments may not be crucial to ERG responsiveness. Here we describe an experiment which quantitatively examines the role of visual pigment remaining in the albino rat retina which has been exposed to continuous low-intensity light. The ERG sensitivity, rhodopsin content and morphological state of the retina were assessed for various exposure durations. Our findings indicate that reductions in visual pigment levels due to constant light persist indefinitely in the dark. Furthermore, the log ERG (b wave) sensitivity varied directly with the rhodopsin content of exposed retinas even as progressive deterioration was apparent. With respect to this log-linear relationship, light damage resembles 'normal' light adaptation due to short term bleaching exposure.

An especially interesting feature of our results is that even extensive damage could be effected by lights which were considerably dimmer than any previously reported. This lighting was provided by 'cool white' fluorescent bulbs with a sheet of translucent, white plexiglass interposed to give a diffusely-illuminated environment of 50–55 lx measured at right angles to the walls of the enclosures. This light level corresponds to that which is found in many indoor environments. Ambient temperature of the cages was constant at $24 \pm 1^\circ\text{C}$ and the rats moved freely within them.

Constant light exposures ranged from 24 to 108 h and were followed by a period of 12 h in darkness. ERGs were then recorded using a Ag–AgCl cotton wick electrode positioned on the cornea of rats which were anaesthetised

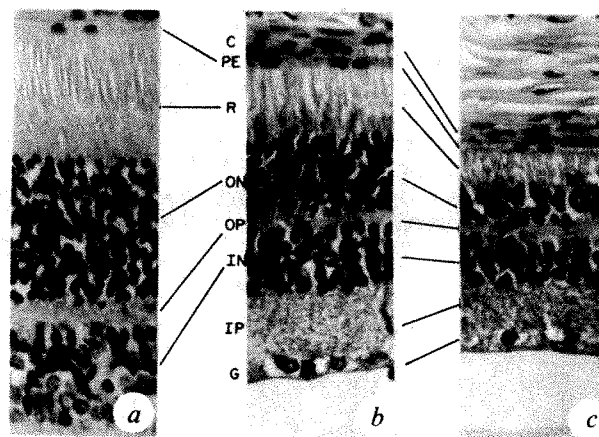


Fig. 1 Light micrographs of transverse sections through albino rat retinas showing different degrees of light damage. *a*, Control retina; *b*, 60-h exposed retina; *c*, 108-h exposed retina. Tissue was fixed in 4% glutaraldehyde and stained with haematoxylin-eosin. Sections were taken from a central region of the retina. C, Choroid; PE, pigment epithelium; R, receptor inner and outer segments; ON, outer nuclear layer; OP, outer plexiform layer; IN, inner nuclear layer; IP, inner plexiform layer; G, ganglion cells. IP and G extend beyond the lower edge of the micrograph of the control retina (*a*). $\times 800$.

with sodium pentobarbital. The indifferent electrode was placed on the unstimulated eye and shielded from stray light. Threshold intensity was determined for a criterion b wave of 25–30 μ V using a 20-ms monochromatic flash of 500 nm. Following electrophysiological measurements, the retinas were excised and used for either histological study or rhodopsin assay in which pigment was extracted with 15% Triton X-100.

Control rats were taken directly from an animal room with a 12-h light, 12-h dark cycle of very dim light. After 12-h dark adaptation, their threshold intensities were measured and equated to 1.0 on a relative scale so that log threshold is equal to 0.0. Rhodopsin content of damaged rats is expressed in this study as a percentage of that found in the controls.

Figure 1 shows the degenerative changes of the rat retina resulting from increasing durations of exposure to constant, low intensity light. With 60-h exposure, a shortening and disorganisation of the outer segment layer can be observed (Fig. 1b). Several cell bodies of the outer nuclear layer were missing and compaction of the retina was evident. No obvious changes in the pigment epithelium were revealed by light microscopy. Rats exposed for 108 h were almost completely lacking in identifiable outer segments (Fig. 1c). The outer nuclear layer was reduced to less than one-third

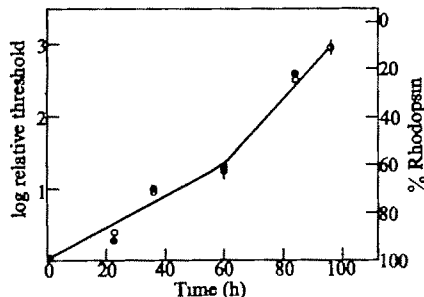


Fig. 2 Time course of sensitivity and rhodopsin content changes with constant exposure to low-intensity illumination. ○, Rhodopsin content; ●, log relative threshold. Standard deviation bars are drawn upon averaged log threshold data points for groups of four animals.

of its original thickness reflecting the loss of many cell bodies; but, of those remaining many seemed to have intact inner segments. In addition, considerably fewer cell nuclei had degenerated in the peripheral retina as compared with the central regions. This distribution of damage has also been observed by previous investigators^{3,4}.

The level of rhodopsin in the retina, as measured after the 12-h dark adaptation period, was continuously lowered with time of exposure to low intensity light. Ordinarily complete regeneration of bleached visual pigment occurs within 2–3 h in the dark⁵. Only 24 h of constant light exposure was sufficient to cause a prolonged reduction in the amount of rhodopsin as compared to the control value. With 108-h exposure, only 11% of the rhodopsin remained. In Fig. 2 it is seen that a steady rise in log b-wave threshold paralleled the depletion of visual pigment. Figure 3 shows the relationship between log threshold and rhodopsin content of the retina for various exposure durations. A linear function between these two variables is obtained which has also been found for retinas bleached (in a conventional manner) with relatively short light exposures^{6,7} and for visual pigment levels reduced by vitamin A deprivation⁸. Thus, our findings demonstrate a third way in which rhodopsin can be varied so that the log ERG threshold depends directly on visual pigment concentration in the retina. It should be noted that the observed rise in threshold due to a reduction in the rhodopsin level is disproportionately higher than what would be expected simply on the basis of the quantal catching ability of the photoreceptors (Fig. 3).

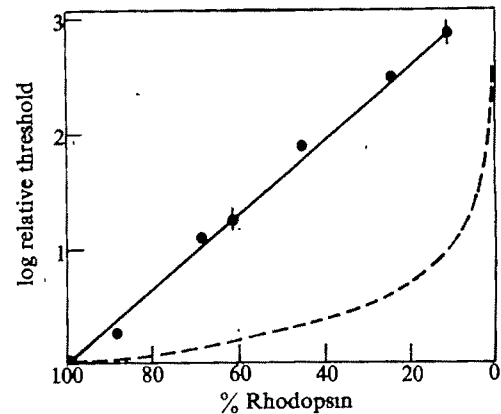


Fig. 3 Relationship between log ERG threshold and reduced visual pigment levels due to constant light exposure. Vertical bars denote the standard deviations of groups of four animals. The dotted line represents the rise in log threshold expected if it were only affected by changes in the probability of quantal absorption by the photoreceptors.

In view of histological changes that are produced by low intensity light exposures, our result implies that even seriously distorted rod outer segments are capable of initiating retinal signals as long as some rhodopsin is present within them. If the cells which generate the b wave can still function at or near their normal capacity following a damaging light exposure, they might only suffer a loss of input in a way that doesn't differ much from 'bleaching' light adaptation. The Müller cells of the inner retina are a likely candidate since they have been implicated as the source cells of the b wave⁹ and have been shown to be spared even after severely damaging light exposures⁴.

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Species specific agglutination of eggs by bindin isolated from sea urchin sperm

SPERMATOZOA adhere to eggs during sea urchin fertilisation^{1,2}. The adhesion is between the acrosome process of the sperm and the vitelline layer covering of the egg³. During the sperm acrosome reaction, the membrane of the extending acrosome process becomes coated with protein derived from the sperm acrosome vesicle^{4,5}. We believe^{4,5}, like others⁶, that the acrosome vesicle protein binds the sperm to the egg by interacting with glycoprotein receptors on the egg vitelline layer^{4,5,7,8}. We have isolated the insoluble contents of the acrosome vesicle and have shown it to be composed of a single protein of molecular weight 30,500 which we have named bindin⁴. In most interspecies inseminations sea urchin sperm fail to adhere to the vitelline layers of foreign eggs and fertilisation does not occur, even though sperm undergo the acrosome reaction in the presence of the foreign eggs⁹. Presumably this failure of gamete adhesion occurs because bindin, on the sperm acrosome process, does not bind to the glycoprotein receptors on the vitelline layers of eggs of another species. We report here the species specific agglutination of unfertilised eggs by isolated bindin. This demonstrates the

preservation of the species specific recognition event between the isolated protein and its receptor which should facilitate the *in vitro* reconstruction and analysis of the molecular mechanism of gamete adhesion.

Gametes were spawned into seawater by injecting 0.5 M KCl into adults. Egg jelly layers were dissolved by a 2-min exposure to pH 5 seawater followed by return to pH 8 seawater. Eggs exhibited 100% normal fertilisation as judged by the elevation of the fertilisation envelope within 60 s after a test insemination. Eggs suspensions used in

agglutination tests were stored at 0 °C no longer than 2 h before being discarded. Insoluble, particulate bindin was isolated from sperm as previously described⁴. After the 13,000g centrifugation the pellet of bindin was transferred to siliconised 30 ml correx glass centrifuge tubes and sonicated with the minimum intensity to achieve complete suspension. The bindin was sedimented three times and resuspended by sonication in 15 ml fresh calcium free seawater pH 5.8 (ref. 4). The final pellet was resuspended in normal seawater, the protein concentration was determined¹⁰ and storage was at 0 °C until use. Eggs were placed in wells of an agglutination titre plate, various amounts of bindin suspension added and the plate agitated by either a reciprocal or a rotary motion at 20 °C.

Figure 1 shows that agglutination of eggs by bindin is species specific. The final concentration of bindin in the agglutination mixture (Fig. 1) was $430 \mu\text{g ml}^{-1}$ for *Strongylocentrotus purpuratus* and $230 \mu\text{g ml}^{-1}$ for *S. franciscanus*. By serially diluting the bindin we determined that for both species $3.5 \mu\text{g ml}^{-1}$ was the minimum bindin concentration needed to agglutinate homologous eggs to the extent shown in Fig. 1. In other words, the concentration of bindin used in this experiment far exceeds the minimum needed to achieve maximum agglutination. That the two bindins do not cross react at high concentrations with eggs of the other species suggests the recognition by bindin of its receptor on the egg surface must be unique to each species. Some loss of specificity occurred as the eggs aged, however. *S. purpuratus* eggs older than 2 h post-spawning sometimes show slight agglutination by *S. franciscanus* bindin, but this was never true for the reciprocal cross mixture. If bindin is partially solubilised in 8 mM urea, 2% mercaptoethanol, then dialysed back into seawater, it still agglutinates eggs but the species specificity is lost. The native structure of bindin seems necessary for the species specificity of agglutination. We have reported⁴ that formaldehyde and glutaraldehyde fixed eggs are agglutinated by bindin. The species specificity of agglutination of aldehyde-fixed eggs is not reliably reproducible for reasons we have not investigated.

The older literature on fertilisation¹¹⁻¹³ stressed that the species specificity of fertilisation is based on the existence of specific 'fertilisins' in the jelly layer surrounding the egg. This hypothesis was recently questioned and re-evaluated by Collins¹⁴. We found that the addition of solubilised egg jelly has no effect on the agglutination of eggs by bindin, and that the species specific attachment of sperm to sea urchin eggs is based on the interaction of a sperm protein, bindin, with the egg vitelline layer. Sperm attachment to the zona pellucida covering of the mammalian egg is also a species specific phenomenon^{15,16} which might involve a similar type of recognition between mammalian sperm bindin and zona bindin receptors. Our work with sea urchins is the first demonstration of the species specific interaction of a protein isolated from sperm with the surface of an animal egg.

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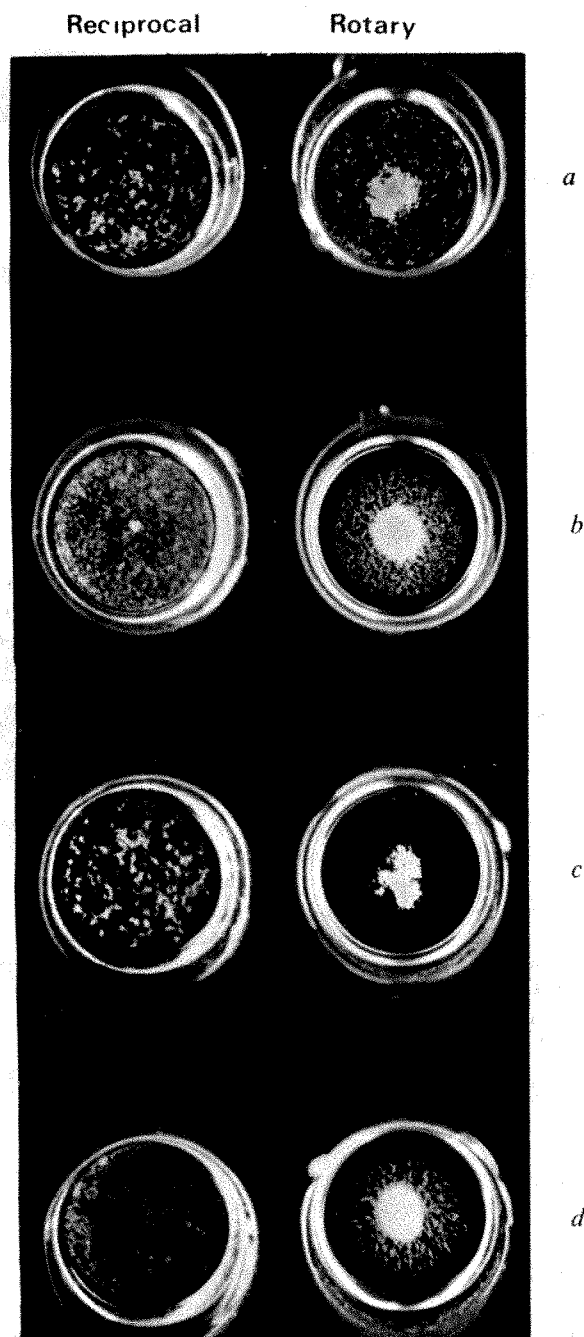


Fig. 1 The agglutination of *Strongylocentrotus purpuratus* and *S. franciscanus* eggs by bindin is species specific. 0.25 ml *S. purpuratus* bindin ($850 \mu\text{g ml}^{-1}$) or *S. franciscanus* bindin ($450 \mu\text{g ml}^{-1}$) was placed in wells (bottom diameter 15 mm) of a Linbro FB-54 agglutination titre plate. 0.25 ml 2% v/v suspension of eggs was added and the plate mixed by either reciprocal or rotary motion with minimum agitation to keep the eggs in suspension. After 2-5 min the agglutination patterns were photographed. Agglutination is easy to discern in homologous mixtures of bindin and eggs. *a*, *S. purpuratus* eggs + *S. purpuratus* bindin = agglutination. *b*, *S. purpuratus* eggs + *S. franciscanus* bindin = no agglutination. *c*, *S. franciscanus* eggs + *S. franciscanus* bindin = agglutination. *d*, *S. franciscanus* eggs + *S. purpuratus* bindin = no agglutination.

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Expression of α -galactosidase in preimplantation mouse embryos

THE most widely accepted model for X-chromosome dosage compensation in female mammals is the single-active X hypothesis (Lyon hypothesis)¹. X-Chromosome dosage compensation probably occurs at about the time of implantation of the embryo into the uterus^{2,3} but it is not known whether this arises by a process of X-chromosome inactivation or by X-chromosome activation⁴. There is now good evidence for the expression of the embryonic genome during preimplantation development⁵⁻¹⁰ and Lyon^{3,2} has reviewed the evidence suggesting that X chromosomes are also expressed during this period. This includes both indirect observations¹¹⁻¹³ and the more direct experiments with the X-linked enzyme hypoxanthine guanine phosphoribosyl-transferase (HPRT) (ref. 10). We have examined the expression of another X-linked enzyme, α -galactosidase¹⁴⁻¹⁶ and report here a 300-fold increase in activity during preimplantation development. This is probably due to the expression of embryonic X chromosomes.

Large numbers of preimplantation mouse embryos at similar stages of development were obtained by artificial insemination (refs 16, 21). Galactosidase activity was assayed in single embryos using the microfluorometric assay technique described for β -glucuronidase^{17,18}. The α -galactosidase assay mixture contained 2×10^{-3} M 4-methylumbelliferyl- α -D-galactopyranoside (Koch-Light), 0.1 M sodium citrate-citric acid (pH 4.2), 0.9% sodium chloride and 0.1% bovine serum albumin.

Embryos were incubated in microdroplets containing 1-8 nl of reaction mixture at either 37 °C or 25 °C for up to 1 h. To avoid depleting the substrate below the optimum concentration, embryos beyond the eight-cell stage were incubated in the reaction mixture for shorter periods (20 min was the shortest incubation time used). The accuracy of the microassay for α -galactosidase was confirmed using a test-tube fluorometric assay^{19,20} for samples of 20-40 embryos. The embryos were added, in less than 5 μ l of phosphate buffered saline, to a reaction volume of 100 μ l which was then frozen and thawed six times and incubated at 37 °C for up to 6 h. The reaction mixture used was the same as for the microassay and at pH 4.2 the reaction was linear with time for 8 h.

The mean α -galactosidase activity per embryo increased 300-fold (from 10^{-14} to 3×10^{-12} mol product per h per embryo at 37 °C) between the two-cell stage (30 h after artificial insemination) and the blastocyst stage (90 h) for C3H/HeHa embryos (Fig. 1). The rate of increase in α -galactosidase activity per embryo also represents a considerable increase in enzyme activity per cell during the initial cleavage divisions (Table 1). This rapid increase in α -galactosidase activity coincides with the increase in activity of HPRT (ref. 10). Other embryonic genes are also expressed at this time⁸ suggesting that the increased α -galactosidase activity is a result of X-chromosome function during early development.

If both X chromosomes of female embryos are active during early preimplantation development X-chromosome dosage compensation would involve the inactivation of one of two active X chromosomes rather than the activation of

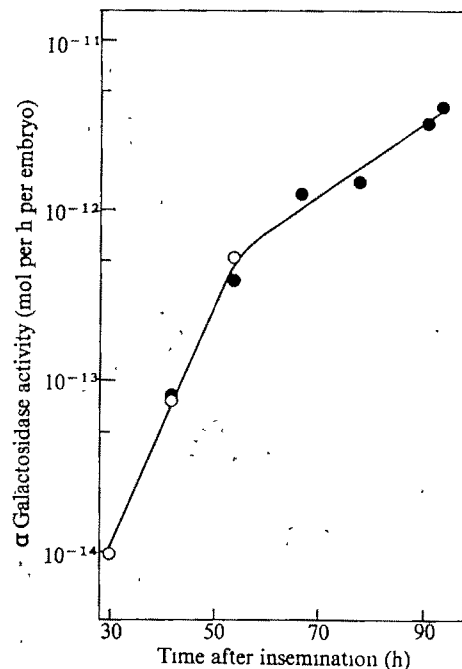


Fig. 1 Increase in mean α -galactosidase activity during preimplantation development of C3H/HeHa mouse embryos. The enzyme activity is expressed as mol of product per h per embryo at 37 °C and results from both microassay (○) and test-tube assay (●) are shown. There is a 300-fold increase in α -galactosidase activity between the 2-cell stage and late blastocyst stage.

one of two inactive chromosomes. It would then be predicted that before X-chromosome inactivation occurs female embryos would have twice the α -galactosidase activity of comparable male embryos, assuming a negligible contribution from the oocyte. The distribution of α -galactosidase activity per embryo for a population of embryos would therefore be expected to be bimodal before X-chromosome inactivation and would become unimodal afterwards.

We have attempted to test this prediction both in order to confirm that both X chromosomes of female embryos are active early in development and to determine whether X-chromosome inactivation occurs before implantation. Bimodal distributions of α -galactosidase activity per embryo were usually obtained for embryos collected at 54 h, and 66 h but in some experiments the distributions were not clearly bimodal. Figure 2 shows the distribution obtained at six time points for our largest experiment in which a possibly bimodal distribution can be seen for the embryos collected at 66 h. Despite our attempts to select embryos at similar stages of development the standard errors for cell counts shown in Table 1 indicate that there is still considerable developmental variation. As the α -galactosidase activity increases very rapidly during this period any

Table 1 Increase in α -galactosidase activity during preimplantation development

Age (h after insemination)	Mean cell no.* ($\pm$ s.e.)	Mean α -galactosidase activity† per cell
30	2	5
42	4	21
54	8	48
66	17.3 ± 0.9	72
78	30.1 ± 2.0	49
90	60.5 ± 4.0	57

*The cell number in 66, 78 and 90 h embryos was determined by the air drying method of Tarkowski²⁰.

†The mean α -galactosidase activity was determined by microassays of 2-cell embryos and test tube assays of older embryos and is expressed as fmol product per cell per h at 37 °C.

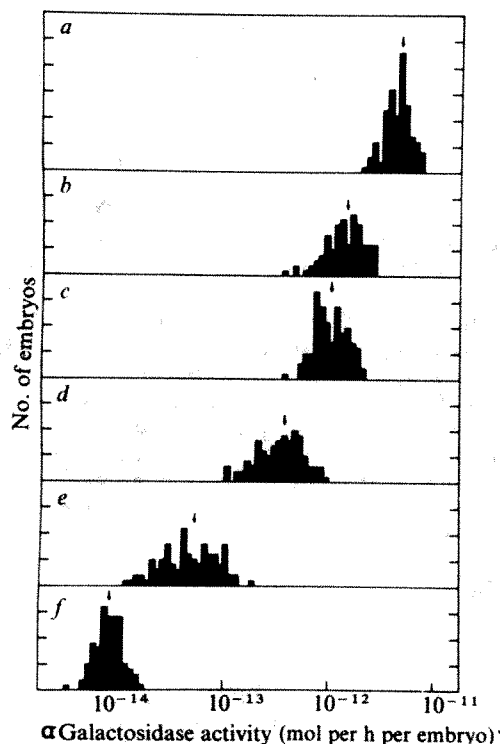


Fig. 2 Distribution of α -galactosidase activity per C3H/HeHa mouse embryo at six time intervals after artificial insemination. Individual embryos were assayed at 25 °C using the microassay and in the experiment illustrated the distribution of α -galactosidase activity per embryo is bimodal for 66-h embryos (morulae). The number of embryos in each activity class is indicated by the vertical scale, marked in intervals of five embryos. The mean enzyme activity for each distribution is indicated with an arrow. *a*, 90 h, mean α -galactosidase activity ($\pm$ s.e.) $4,523 \pm 124$ fmol product per h per embryo at 25 °C ($n = 104$); *b*, 78 h, $1,504 \pm 62$ ($n = 91$); *c*, 66 h, $1,094 \pm 38$ ($n = 111$); *d*, 54 h, 386 ± 19 ($n = 99$); *e*, 42 h, 56.1 ± 3.4 ($n = 99$); *f*, 30 h, 8.7 ± 0.3 ($n = 95$).

developmental variation among embryos of the same age would contribute to variation in enzyme activity per embryo and tend to mask any underlying bimodal distribution.

The striking increase in α -galactosidase strongly suggests that X chromosomes are expressed in preimplantation mouse embryos as early as the four cell stage. We have not yet been able to confirm that female embryos have higher levels of α -galactosidase activity than males during preimplantation development. However, the indications of bimodal distributions of α -galactosidase activity per embryo suggest that both X chromosomes are expressed in female preimplantation embryos by the eight-cell to morula stage. These results support the view that X-chromosome dosage compensation occurs by the inactivation of one of two active X chromosomes rather than by the activation of one of two inactive X chromosomes.

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Low specific activity of rare allozymes of α -glycerophosphate dehydrogenase in *Drosophila*

ELECTROPHORETIC surveys of *Drosophila* species¹ show that many gene-enzyme systems are essentially monomorphic. Very often, however, one or more populations may contain rare alleles, that is, at frequencies less than 5%. Are these alleles selectively neutral and rare because of historical accidents² or are they deleterious and maintained in populations only through mutation? The dimeric enzyme α -glycerophosphate dehydrogenase (α GPDH) is a good example of an essentially monomorphic *Drosophila* gene-enzyme system. Out of the 101 species of *Drosophila* surveyed thus far for electrophoretic variation of α GPDH (refs 3-5), only *D. melanogaster* is polymorphic. This polymorphism seems to be maintained by selection with temperature being, or strongly correlated with, the selective agent⁶⁻⁸. Microcomplement fixation experiments indicate that this enzyme is evolving slowly among *Drosophila* species and has accepted relatively few changes in its structure⁹. We propose that the rare alleles of the α GPDH locus found in some species (see ref. 5) may be at low frequencies because the products of these alleles (allozymes) are catalytically less efficient than their 'wild-type' counterparts.

To test this hypothesis, the specific activities of all the available rare electrophoretic variants (allozymes) were compared with the specific activities of the common allozymes in six species of *Drosophila*. Two other species, *D. nasuta* and *D. birchii*, for which population survey data are not available were also examined. The 'rare' alleles in these species were those found at low frequencies in laboratory stocks. Although there was an insufficient number of flies homozygous for the rare alleles to determine specific activities for the purified allozymes directly, there is a way to determine them indirectly. α GPDH is a dimeric enzyme and in α GPDH^A/ α GPDH^B heterozygotes the A : A, A : B and B : B dimers can be separated electrophoretically. The activities of each of these allozymes can then be measured densitometrically after staining for α GPDH activity. If it is assumed that each allele produces an equal amount of protein and that the monomers associate randomly, then the relative activities of the A : A and B : B homodimers in the heterozygous flies, in fact, represent the relative specific activities of those two forms of α GPDH (Fig. 1).

The critical assumption that the monomers associate randomly is supported by three facts. First, *in vitro* subunit hybridisation experiments using purified α GPDH from eight species of *Drosophila* have shown that the dissociated monomers of these different α GPDHs reassociate randomly¹⁰. Second, theoretical calculations on the effect of single amino acid substitutions in the subunit contact region of this molecule strongly suggest that they would have little discernible effect on the ratio of dimers in heterozygotes¹⁰. Third, only a minority of the many rare variants of haemoglobin affect the subunit contact regions of this molecule¹¹. Thus, few, if any, of the rare allozymes of α GPDH are expected to be affected in the subunit contact

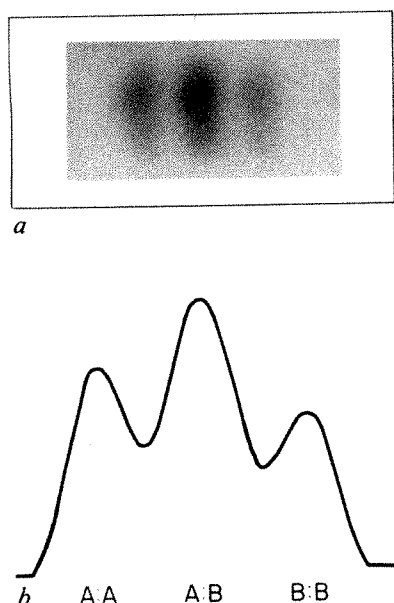


Fig. 1 Electrophoretic separation of α GPDH allozymes from *D. paramelanica* heterozygous at the α GPDH locus. *a*, Cellulose acetate membrane stained for α GPDH activity after electrophoresis. Electrophoresis was carried out in a Beckman Microzone Cell at 300 V for 1 h, in a buffer consisting of one part Tris-barbital-sodium barbital buffer (0.025 ionic strength, pH 8.8) and one part Tris-HCl buffer (0.1 M, pH 8.3). The membranes were stained for 10 min at 20 °C in 25 ml of 0.05 M Tris-HCl buffer, pH 8.3, which contained 15 mg NAD⁺, 5 mg MTT tetrazolium, 3 mg phenazine methosulphate, 200 mg α -glycerophosphate, and 375 mg agar. For the staining time used, the deposition of dye is linear and therefore proportional to enzyme activity (G.E.C., unpublished results). *b*, Densitometer scan of the stained membrane. The area under the B:B peak compared with that under the A:A peak is taken as the relative specific activity of the B:B homodimers.

region of this molecule. It is also doubtful that such effects would alter the ratio of dimers in heterozygotes.

Table 1 lists the observed activity ratios for α GPDH in heterozygotes, together with those expected on the basis of the relative specific activities calculated as outlined. Although there are no gross departures from expectation in these values, there are consistent deficiencies for the

observed values for the heterodimers. The most likely explanation is that the heterodimers do not have specific activities exactly intermediate to those of the respective homodimers, as assumed for calculating the expected ratios in Table 1, but rather have specific activities slightly lower. Thus, in spite of the approximate nature of the calculation, we feel that this is a valid means of estimating the relative specific activities of rare allozymes of α GPDH.

The activities of nine rare allozymes (the B:B allozymes) thus calculated (Table 1) are all lower than their wild-type counterparts (the A:A allozymes). In addition, although the electrophoretic separation of the dimers in α GPDH^A/ α GPDH^B heterozygotes of *D. immigrans* was not sufficient to make a densitometric tracing, it could be seen that the B:B homodimer was not as intensely stained as the A:A homodimer. From these results it might seem that α GPDH^B of *D. birchii* might be a rare allele in natural populations and that α GPDH^B in *D. nasuta* might be polymorphic. Surveys of α GPDH variation in populations of these two species would represent a further test of this hypothesis.

It is also interesting that homozygotes for the rare alleles in some species have low viabilities, reduced fertility and/or longer developmental times. For example, in *D. putrida*, α GPDH^B/ α GPDH^B homozygotes were obtained from matings between heterozygotes, but no adult flies ever eclosed from crosses between these α GPDH^B homozygotes. In *D. tripunctata*, homozygotes for the rare allele were viable but their egg-adult developmental period was 3–4 d longer than wild-type flies raised in the same conditions. The same was true in *D. algonquin*, except that the developmental time of the α GPDH^B homozygotes was only increased 2–3 d. Although the possibility that these effects are due to other closely linked deleterious genes cannot be completely ruled out, it is unlikely that this is the case because none of the α GPDH^A/ α GPDH^A stocks established from single pair matings ever exhibited these characteristics.

The total activity of α GPDH measured on cellulose acetate membranes is virtually the same for α GPDH^A/ α GPDH^A and α GPDH^B/ α GPDH^B homozygotes, although the two homodimers in a heterozygote do not have the same relative activities. This is true even in those species

Table 1 Ratio of α GPDH dimers in heterozygotes and relative specific activities of α GPDH produced by rare alleles

Species	Allele*		Ratio of dimers (% total activity)			Relative specific activity† (B:B against A:A homodimers) (%)	
			A:A	A:B	B:B	(Range)	
<i>D. nasuta</i> ¶	B	Observed (s.e.)	28.3 (2.3)	44.7 (2.9)	27.0 (1.8)	96	(90–104)
		Expected‡	25.5	50.0	24.5		
<i>D. robusta</i>	B	Observed (s.e.)	29.7 (2.5)	43.9 (4.8)	26.4 (2.6)	89	(79–100)
		Expected‡	26.5	50.0	23.5		
<i>D. robusta</i>	C	Observed (s.e.)	32.1 (5.5)	39.3 (10.4)	28.6 (5.5)	89	(81–95)
		Expected‡	26.5	50.0	23.5		
<i>D. affinis</i>	B	Observed (s.e.)	29.0 (3.0)	49.3 (3.3)	21.7 (0.8)	75	(66–81)
		Expected‡	28.6	50.0	21.4		
<i>D. paramelanica</i>	B	Observed (s.e.)	31.3 (2.2)	45.6 (3.1)	23.1 (1.1)	74	(64–79)
		Expected‡	28.7	50.0	21.3		
<i>D. birchii</i> ¶	B	Observed (s.e.)	33.1 (2.1)	43.8 (3.2)	23.1 (1.5)	70	(55–80)
		Expected‡	29.4	50.0	20.6		
<i>D. algonquin</i>	B	Observed (s.e.)	36.0 (3.1)	41.0 (4.8)	23.0 (2.2)	64	(58–66)
		Expected‡	30.5	50.0	19.5		
<i>D. tripunctata</i>	B	Observed (s.e.)	36.5 (4.0)	43.1 (5.8)	20.4 (1.9)	56	(54–60)
		Expected‡	32.0	50.0	18.0		
<i>D. putrida</i>	B	Observed (s.e.)	41.7 (2.1)	47.7 (3.1)	10.6 (1.9)	20	(15–32)
		Expected‡	41.7	50.0	8.3		

*The common allele in each species is designated A. Except for ¶, the remaining alleles occurred in natural populations at frequencies < 0.02 (refs 6,7).

¶The alleles for these species were found in laboratory stocks. Their frequency in wild populations is not known.

‡The expected ratio was calculated using the relative specific activities of the A:A homodimer as $p = 1$, and that of B:B as $q < 1$, (see †). Then the expected ratio is $A:A = p/2(p+q)$; $A:B = (p+q)/2(p+q)$; and $B:B = q/2(p+q)$.

†The method for determining relative specific activities is discussed in the text and Fig. 1. Each recorded value is an average of 4–8 separate determinations.

having a rare allozyme of rather low relative specific activity, for example *D. putrida*. Berger¹² pointed out that the activity level of an enzyme may be an important factor in the maintenance of protein polymorphism. Thus, an individual which has to make more of a less active allozyme may suffer from the biochemical cost of increased synthesis. In this regard, α GPDH is surely a major protein component of the adult fly, for it is homogeneous after only some 500-fold purification of crude extracts of *D. melanogaster*¹³.

Although additional biochemical characterisation of these allelic products is needed, the preliminary results indicate that the enzymes made by the rare alleles at the α GPDH locus are molecules with lowered enzymatic efficiency (although tightly linked *cis* regulatory sites can be associated with different XDH allozymes in *D. melanogaster*)¹⁴. It therefore seems reasonable that these alleles are rare because they are selected against in natural populations. Whether this holds for rare alleles in other *Drosophila* gene-enzyme systems remains to be determined.

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Phenotypic characterisation of a unique non-T, non-B acute lymphoblastic leukaemia cell line

MANY human lymphoblastoid cell lines have been established from subjects with different haemopoietic disorders as well as from normal individuals¹⁻³. In most cases the inability to identify the precise origin of cells which have been established has limited the usefulness of these lines, especially those derived from leukaemic donors. Although there are a few important exceptions to this generalisation (2, 3, 4 and see below) it is significant that no lines have been established from the common form of acute lymphoblastic leukaemia (ALL) which is usually referred to as 'null' cell or 'non-T, non-B' ALL (refs 5-7). We describe here the establishment of a lymphoid cell line, Reh, derived from an acute lymphocytic leukaemia where the leukaemic identity of established cells is supported by kinetic data, chromosome markers and detection of leukaemia associated antigens and enzymes.

Reh cells do not contain detectable Epstein-Barr virus (EBV), either by the nuclear antigen (EBNA) test or by nucleic acid hybridisation (to be published elsewhere). This line was initiated from the peripheral blood of a 15-yr-old girl in relapse with 100% lymphoblasts in her bone marrow and 146,000 white blood cells (mm³) with more than 95% lymphoblasts in her peripheral blood. Clinical and haematological features including morphology and histochemistry of the leukaemic blasts were characteristic of ALL.

Details of culture and karyotype techniques have been described previously⁸. Karyotypes were obtained and studied at the time of seeding the culture, and after establishment without addition of PHA. Fresh leukocytes consisted of populations which were cytogenetically different, one normal: 46, XX, the other abnormal: 45, XX, -2B, +1C (Fig. 1a).

Denaturation techniques have suggested that the two missing B are possibly one number 4 and one number 5 and further indicated that the extra C is not a true C, but probably reflects a structural rearrangement. Early in the course of establishment, chromosome examination revealed that the selection of the abnormal population had

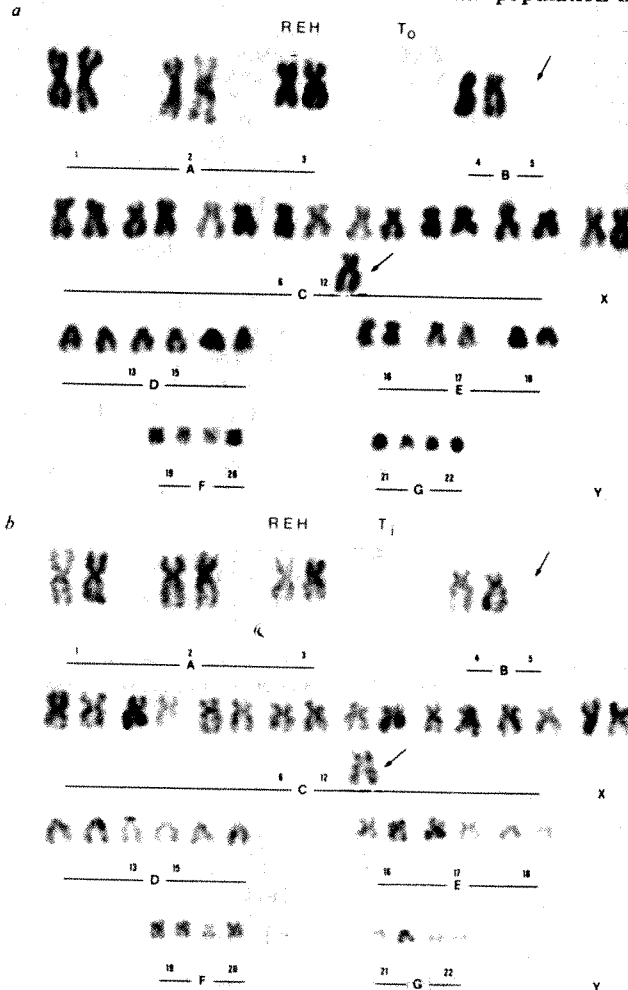


Fig. 1 Chromosome preparations from Reh cells. To, Fresh cells. Ti, Established line. See text for interpretation.

occurred since all the cells showed the 45, XX, -2B, +1C karyotype (Fig. 1b). Disappearance of the normal population was confirmed by further sequential examinations. Analysis with banding techniques is necessary to establish the precise nature of Reh chromosomal characteristics. The results so far, however, indicate clearly that the cells established *in vitro* belong to the same cytogenetically abnormal clone as the patients' uncultured leukaemic cells.

In contrast to the situation found with normal B-cell lines, the Reh cells started to divide at the beginning of the culture. This can be seen in Table 1 from the percentage of mitosis and from the cell uptake of tritiated thymidine (³HTdr). These observations suggest that abnormal leukocytes were immediately able to proliferate in the culture medium and overcome the cytogenetically normal population.

The fresh Reh cells and the Reh line when established were examined for reactivity with a small panel of membrane markers. The initial cells from the patient and the

Table 1 Tritiated thymidine uptake and percentage of mitosis during the establishment of the Reh cell line.

Time in culture (d)	% ³ HTdR labelled cells	% Mitosis
0	14.1	0.13
7	11.4	0.12
14	10.6	0.23
26	9.4	0.17
42	12.1	0.32
54	20.9	0.28
67	13.2	0.45
77	17.5	0.85
96	19.2	0.90
201	22.9	1.10

The percentage of DNA synthesis was determined by the 'dipping' autoradiographic technique. After incubation for 1 h at 37 °C with ³HTdR (1 µCi ml⁻¹ TMM 79 B, CEA, France) the cells in suspension were centrifuged (5 min at 800 r.p.m.), spread and fixed 10 min in methanol. The slides were dipped in Ilford K2 emulsion at 45 °C, dried and set in the dark at 4 °C. Exposure time was 7 d. After development (Kodak D19B, AL4) the slides were rinsed under water, dehydrated for 45 min with ethanol 70 °C and coloured with Giemsa dye. The labelling index was determined by counting 1,000 cells. The percentage of mitosis was measured on 10,000 Giemsa-stained cells before establishment and on 2,000 cells after establishment.

established cell *in vitro* were negative for sheep erythrocyte rosette formation and surface immunoglobulin and could therefore be considered to be of the common type of ALL (that is 'null' ALL or 'non-T, non-B' ALL). Soon after establishment a small proportion of the Reh cells seemed to have cell surface C3 binding sites as determined by the EAC rosette assay²; however these were no longer detectable after a few months of culture.

The Reh line has now been maintained in culture for 2 yr and during this time has been used for active immunotherapy. Recently several new leukaemic cell markers have become available^{6,10,11} and so a more extensive phenotyping

of the Reh line has now been carried out. Table 2 shows the results of this analysis in which the phenotype of Reh is compared with that observed in a large series (400) of ALL cases^{6,12}. The phenotype profile of Reh is indeed essentially identical to that of the common form of ALL. The crucial markers here are the anti-ALL sera¹³, anti-p28,33 serum^{12,14} and the enzymes hexosaminidase (intermediate peak isoenzyme)¹² and terminal deoxynucleotidyl transferase¹⁵ since these together constitute a phenotype unique to non-T, non-B ALL¹². Figure 2 illustrates the reactivity of Reh cells with anti-ALL. Absorption of anti-ALL serum with Reh cells removes all binding activity against fresh ALL cells (M.G., unpublished) indicating complete cross-reactivity of the ALL antigen on Reh and non-T, non-B ALL.

Several cell lines have been established from ALL by other investigators. With rare exceptions, however, these have either been EBV positive B cells (presumably derived from the patient's normal B cells) or in cases of Thy or T-like ALL, they have been EBV negative T-cell lines^{16,17}. The latter are presumably leukaemic cell derivatives and in some cases this has been confirmed by chromosome markers. An exception to this generalisation is the establishment of the line NALM-1 from a child with Philadelphia positive CML in blast crisis⁴. The membrane phenotype of this line is the same (with respect to those markers tested) as Reh including the expression of the ALL antigen⁴. This observation accords with recent studies indicating that some cases of CML and non-T, non-B ALL may originate from a common target cell¹⁸. In general it has proved impossible to establish non-T, non-B ALL cell lines (J. Minowada, personal communication). The only reported exceptions in addition to Reh and the Ph¹ positive NALM-1 are two lines of non-T, non-B ALL established from the cells of patients in relapse¹⁹. It may be that the only non-T, non-B ALL type cells which have a capacity

Table 2 Membrane marker and enzyme assays on the Reh line compared with non-T, non-B ALL cells

Markers	Reh line	Non-T, non-B ALL	Ref.*
Antisera			
R. anti-ALL, R6†	+	+	13, 24, 25
R. anti-ALL, R73	+	+	
R. anti-ALL, R74	+	+	
R. anti-ALL, R8 F(ab') ²	+	+	
R. anti-p28, 33 'Ia-like antigen‡	+	+	14, 25 25, 26
R. anti-monkey thymus F(ab') ² sheep anti-human Ig§	—	—	
Sheep anti-kappa light chain	—	—	27, 28
Sheep anti-lambda light chain	—	—	
R. anti-'myeloid' antigen R. 101	—	—	
antigen R. 102	—	—¶	
Rosette assays			
E sheep	—	—	29, 30
E mouse: no neuraminidase	—	—	
E mouse: plus neuraminidase	+(39%)	—(?)††	31 31 31
E (Ox) IgM	—	—	
E (Ox) IgM + C	—	—	
E (Ox) IgG	—	—	
Enzymes			
Hexosaminidase 'I' isoenzyme	++	+ / + + +	12, 32
Terminal deoxynucleotidyl transferase	360**	1.5–190 (mean 57)	15, 33

*Reference for reagents, assay methods, and results on uncultured ALL cells.

†R6, 73, 74, 8 are for different rabbit anti-ALL sera. They are extensively absorbed with normal tissues and non-ALL leukaemias.

‡A gift from Dr Ken Welsh (Queen Victoria Hospital, East Grinstead).

§A gift from Dr I. Chantler (Burroughs-Wellcome).

¶Very faint staining with both anti-kappa and anti-lambda was observed on all Reh cells. We interpret this as minimal binding which is probably nonspecific.

¶Rabbit anti-acute myelo-monocytic leukaemia cell serum absorbed with lymphoid cells. This serum defines a myeloid differentiation antigen which is absent from normal and leukaemic lymphoid cells (Roberts, M. and M.F.G., in preparation).

**Units per 10⁶ cells. 1 unit is 1 nmol⁻¹ of nucleotide polymerised at 37 °C (Ganeshaguru, K., Hoffbrand, G. V., Janossy, G. and M.F.G., in preparation). E, erythrocyte. C, complement. Fresh mouse serum used for this complement receptor assay.

††Out of 6 tested.

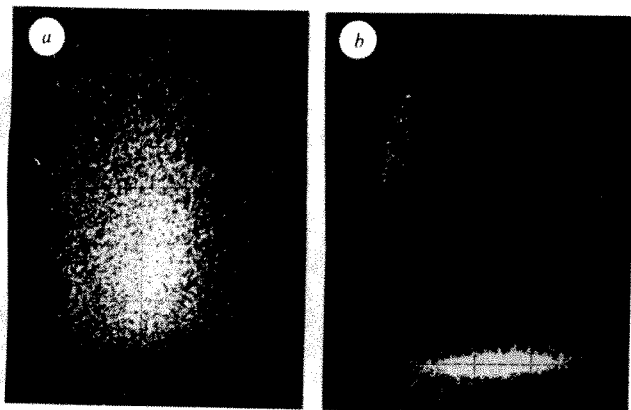


Fig. 2 Fluorescence activated cell sorter (FACS) analysis of anti-ALL binding by Reh line. Vertical axis: relative fluorescence intensity. Horizontal axis: relative cell size. *a*, Reh cells stained with rabbit anti-ALL followed by fluorescein labelled goat anti-rabbit Ig. *b*, Reh cells treated with normal rabbit serum followed by fluorescein labelled goat anti-rabbit Ig. See ref. 6 for details of FACS and fluorescence staining methodology.

for unlimited growth *in vitro* are those which are aneuploid with major chromosomal alterations. These changes may occur at the onset of the disease and affect all members of the leukaemic clone or more frequently may arise after treatment in a subclone (perhaps in Ph negative ALL). The ability of these exceptional ALL or CML blast crisis cells to grow *in vitro* may be a correlate of their growth patterns *in vivo* since relapsed ALL is often more drug resistant than previously untreated ALL (ref. 20) and Ph⁺ positive disease, even when presenting with an ALL phenotype, has a worse prognosis than Ph⁻ negative ALL in the same age group^{18, 21, 22}.

The availability of cell lines which are clearly derived from the common form of lymphoblastic leukaemia may be of considerable value in further investigations on the nature of leukaemia-associated antigens, and the block to further differentiation which presumably exists in these cells. The Reh line has been used extensively for active immunotherapy of ALL patients in Villejuif²³. It is possible that the therapeutic efficacy of Reh might be different from that of fresh allogeneic ALL cells. The ALL associated antigen might in combination with allogeneic histocompatibility antigens provide a suitable target for the patients' immune system. We are currently testing sera from treated patients to see if antibodies are present against the ALL antigen which the rabbit antisera recognise.

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Human B cell, T cell and null cell leukaemic cell lines derived from acute lymphoblastic leukaemias

CONTINUOUS culture of human leukaemic cells is still a difficult task, although several leukaemic cell lines have been derived from patients with acute and chronic myelogenous leukaemia^{1,2} and acute lymphoblastic leukaemia (ALL) (refs 3–6). We describe here B-cell, T-cell and null-cell leukaemic cell lines newly established from three patients with ALL.

Leukaemic cells separated by dextran sedimentation from peripheral blood or bone marrow were resuspended in growth medium at a concentration of 1×10^7 cells per ml and dispersed into 35-mm Petri dishes (2.5 ml per dish). The cultures were incubated at 37 °C in a humidified atmosphere of 7.5% CO₂ in air. Two kinds of growth media were used: medium I consisted of RPMI 1640 supplemented with 20% human cord serum (HCS) and medium II of RPMI 1640 supplemented with 20% foetal calf serum (FCS). Partial medium changes were done twice a week. After cell line establishment, subcultures were made usually once a week.

Cells were examined for receptors for sheep erythrocytes (E), Fc fragment of IgG (EA) and complement (EAC). They were also tested for surface immunoglobulin (Ig) by direct immunofluorescence with FITC-conjugated goat antisera (Behringwerke) monospecific for human IgM, IgG, IgA and light chains. All these assays were performed as described by Jondal and Klein⁷. Epstein-Barr virus (EBV)-determined nuclear antigens (EBNA) was tested for by anti-complement immunofluorescence⁸. Karyotypes were analysed as described previously⁹.

A B-cell line (BALL-1) was derived from peripheral blood of a 75-yr-old man who died 1.5 months after the onset of ALL. Culture was initiated on 15 January 1976

and established only in medium I 1 month later. The peripheral leukocyte count at the beginning of culture was 25×10^4 per cm^3 with 83% lymphoblasts. Both peripheral lymphoblasts and BALL-1 cells were virtually 100% surface Ig-positive for two heavy chain classes IgM and IgA. The light chain was of the lambda type. Cells from both sources contained 36–60% EA and EAC rosetting cells but neither of them formed E rosettes. Autopsy on this patient revealed no lymphadenopathy or tumorous masses.

A T-cell line (TALL-1) was established from bone marrow of a 28-yr-old man who developed the terminal leukaemic phase of lymphosarcoma. Marrow cells cultured on 13 April 1976 grew equally well in both medium I and II after 1 month. The marrow at the time of culture contained 94% lymphoblasts. The majority of marrow lymphoblasts and TALL-1 cells formed E rosettes but neither of them bore surface Ig.

A null-cell line (NALL-1) was obtained from peripheral blood of a 14-yr-old boy who relapsed after a 3-month remission. Culture was started on 16 March 1976 and established only in medium I 2 weeks later. At the time of culture initiation, the peripheral leukocyte count was 18.8×10^4 per cm^3 with 98% lymphoblasts. Neither peripheral lymphoblasts nor NALL-1 cells demonstrated E rosette formation or surface Ig. Receptors for EAC but not for EA were detected on a small percentage (less than 8.1%) of NALL-1 cells, however.

Cells from BALL-1, TALL-1 and NALL-1 grew in single cell suspension with a doubling time of 60–90 h and were morphologically identical to the original lymphoblasts of the respective donor. These cell lines were all repeatedly negative for EBNA when tested by us and in the laboratory of Dr Yoriko Hinuma, and hence considered EBV genome-free.

Karyotypic analysis was performed on these cell lines between 7 and 9 months after culture initiation. Fifty metaphases were studied from each sample and several selected metaphases were further analysed by the Giemsa banding technique. BALL-1 had a pseudodiploid mode with four characteristic markers (one each of large telocentric and metacentric chromosomes and two medium-sized telocentric chromosomes). Two metaphases were obtained from peripheral lymphoblasts from the BALL-1 donor, both of which were pseudodiploid. The chromosomes of the TALL-1 line were all in the hypertetraploid region, ranging from 95 to 101. No chromosome preparations were made directly from the donor of the TALL-1 line. NALL-1 had a hypodiploid mode of 43 with a minute marker and missing sex chromosomes. Peripheral lymphoblasts from the NALL-1 donor yielded three analysable metaphases, which all showed 43 chromosomes and the minute marker observed in the NALL-1 line.

When BALL-1, TALL-1 and NALL-1 were stained with rabbit anti-human thymocyte serum by indirect membrane immunofluorescence, only TALL-1 cells were fluorescent, indicating the absence of thymus-related antigen on BALL-1 and NALL-1 cells. The anti-thymocyte serum had been extensively absorbed with 'normal' lymphoblastoid cell lines, chronic lymphocytic leukaemia cells and normal peripheral leukocytes, and known to react specifically with MOLT-4 (leukaemic T-cell line) and thymocytes. Furthermore, BALL-1 and NALL-1 were found to stimulate allogeneic lymphocytes in one-way mixed lymphocyte reactions whereas TALL-1 was non-stimulatory.

Continuous multiplication of B-cell and null-cell leukaemic cells in medium I in contrast to their rapid degeneration in medium II suggests that HCS is more growth-supporting than FCS for certain human ALL cells. On the other hand, T-cell lymphoblasts grew equally well in both medium I and II; this is consistent with the establishment of T-cell ALL lines in other laboratories in

medium containing FCS (refs 3–6). Both BALL-1 and NALL-1 initially established in medium I were then adapted to grow in medium II, although they tended to multiply better in medium I than in medium II. Another advantageous point of the supplemental use of HCS is that antibody present in HCS against EBV capsid antigen (VCA) would suppress transformation of coexisting normal B lymphocytes by EBV (ref. 10). The pooled HCS that we used in the present experiment had an anti-VCA titre of 1:160.

The assumption that all of BALL-1, TALL-1 and NALL-1 originated from leukaemic cells is based on the findings that they uniformly lack the EBV genome, have morphology and surface characteristics identical to those of the leukaemic cells of the corresponding donor and have various chromosome abnormalities including those found in the pre-culture lymphoblasts. Therefore, these cell lines are distinct from EBV-carrying lymphoblastoid cell lines of normal B-lymphocyte origin¹¹.

A question may still be raised as to the possible derivation of the BALL-1 line from normal B lymphocytes of the leukaemic patient, even though it is EBV-negative. This would be unlikely, however: rare cases of B-cell ALL do exist^{12,13}; EBV-negative lymphoblastoid cell lines have never been established from normal tissues¹¹; and in addition to the characteristics described above; BALL-1 cells have produced serially transplantable lethal tumours in newborn hamsters treated with anti-lymphocyte serum (unpublished data).

Minowada *et al.*¹⁴ have established a Ph¹ chromosome-positive non-T non-B (null-cell) line from a child with chronic myelogenous leukaemia in blast crisis. Clearly, this cell line is different from NALL-1 with respect to the Ph¹ chromosome and its derivation. The B-cell and null-cell leukaemic cell lines we describe here are unique and, used together with T-cell lines, should contribute to further understanding of human lymphocyte subpopulations in normal and leukaemic states.

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In vitro immunisation against human tumour cells with bacterial extracts

LYMPHOCYTES from normal persons can become immunised to a variety of tumour cells by exposure to tumour cells *in vitro*¹⁻³. Since close antigenic relationships between micro-organisms and some tumour cells have been demonstrated⁶⁻⁹, studies were carried out to see if bacterial antigens could similarly immunise lymphocytes from normal persons *in vitro* against some tumour cells. The experiments reported here indicate that not only is this possible, but that the cytotoxicity of bacterial immunised lymphocytes is sometimes greater than when lymphocytes are sensitised by intact tumour cells.

Soluble bacterial extracts were prepared from the Glaxo strain of *Mycobacterium bovis* (BCG), *Listeria monocytogenes* (LM), *Escherichia coli* (EC), and *Staphylococcus aureus* (SA), as previously described¹⁰ and are referred to as BCG-SS, LM-SS, EC-SS, and SA-SS, respectively. Their nitrogen (N) concentrations were determined¹¹ and they were passed through a Millipore filter before use.

Breast tumour cells that originated from the pleural effusion of a patient with breast carcinoma were obtained from Dr G. Seman, MD Anderson Hospital, Houston, Texas¹². Malignant melanoma cells (RPMI-7932 and RPMI-8322) were provided by Dr G. E. Moore, Denver General Hospital, and are referred to as melanoma-1 and melanoma-2. Melanoma-1 originated from a pleural effusion and melanoma-2 was established from a metastatic solid tumour lesion. Tumour cells were maintained in tissue culture¹.

Peripheral blood lymphocytes were obtained from 12 normal tumour-free persons. Lymphocytes were then isolated and purified over Ficoll-Hypaque⁴. The purified lymphocytes contained less than 5% polymorphonuclear leukocytes and 5-10% monocytes. These were sensitised with mitomycin C treated tumour cells as previously described⁴ or with various concentrations of the bacterial extracts. The cytotoxicity of *in vitro* sensitised cells was then measured by a ⁵¹Cr release assay¹⁻⁴. Results were compared with the capacity of the same cells to kill normal lymphoblastic target cells. In only one case (donor number 4) was there as much as 25% specific ⁵¹Cr release from normal lymphoblasts (Table 1). Accordingly, ⁵¹Cr release of 25% or more was chosen in this report as the criterion for specific cytotoxicity.

BCG-sensitised lymphocytes from eleven donors caused a greater release of ⁵¹Cr (28% to 78%) from melanoma-1 cells than from the control lymphoblasts (-2% to 21%). Melanoma-2 cells were killed by BCG-immunised lymphocytes from two of three donors and breast tumour cells were killed by BCG-immunised lymphocytes from five of eleven donors. LM-sensitised cells from all seven donors were similarly cytotoxic to melanoma-1 cells and LM-sensitised cells from five of seven donors were cytotoxic to breast tumour cells. In contrast, lymphocytes from only three of seven donors treated with SA-SS became cytotoxic to melanoma-1, and two of seven became cytotoxic to breast tumour cells. Lymphocytes from four donors were treated with EC-SS and none of these developed appreciable cytotoxicity against either melanoma or breast tumour cells. Generally, LM-SS appeared on a nitrogen basis to be more immunogenic than BCG-SS. In some cases, as little as 2 µg N of LM-SS effectively immunised normal lymphocytes. Data from individual experiments where lymphocytes from normal donors were treated with equivalent amounts of BCG, LM, and EC are presented in Fig. 1.

Lymphocytes from some of the donors that were pre-treated with phytohaemagglutinin (PHA), pokeweed mito-

gen (PWM), and concanavalin A (con A) were also treated as effector cells. PHA treated lymphocytes from four of ten persons became cytotoxic to melanoma-1 cells and cells from three of five donors that were PWM treated became cytotoxic to melanoma-1 and one of these also to breast tumour cells. Lymphocytes from one of six donors treated with con A became cytotoxic to melanoma-1 and none to breast tumour cells. PHA treated lymphocytes have

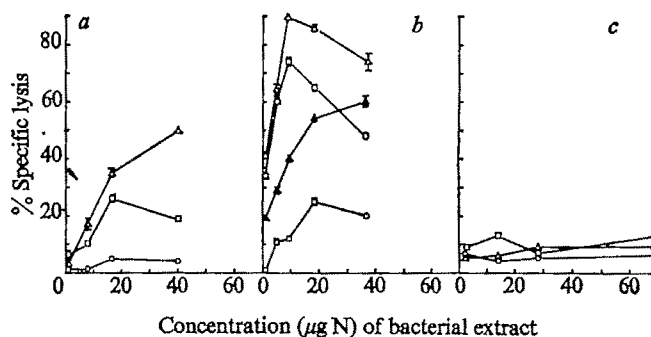


Fig. 1 *In vitro* immunisation of lymphocytes against human tumour cells with bacterial extracts. Lymphocytes were sensitised with different concentrations of: a, BCG extract (BCG-SS); b, *Listeria monocytogenes* extract (LM-SS); c, *Escherichia coli* (EC-SS). Sensitised lymphocytes were tested against target cells: Δ, melanoma-1 tumour cells; ▲, melanoma-2 tumour cells; □, breast tumour cells; ○, normal lymphoblasts. % Specific lysis of ⁵¹Cr-tumour cells was measured. Only standard deviations greater than 1% are indicated.

previously been shown to be cytotoxic to some tumour cells¹³⁻¹⁶. The degree of cytotoxicity observed in this study was relatively less and more variable with the mitogens tested than those produced by the bacterial extracts, however, especially against breast tumour cells. This would tend to minimise the possibility that the cytotoxicity initiated by the bacterial extracts was entirely due to mitogenic effects.

If *in vitro* sensitisation of lymphocytes by bacterial

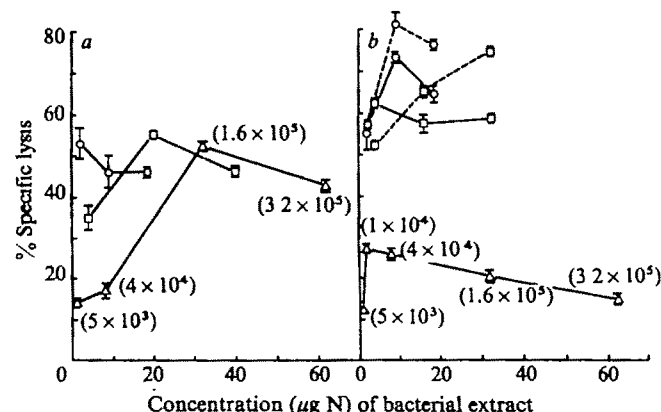


Fig. 2 *In vitro* immunisation of lymphocytes with tumour cells or bacterial extracts. a, Lymphocytes were sensitised with breast tumour cells (Δ), BCG-SS (□), or LM-SS (○) and tested against breast tumour cells. Number of immunising tumour cells shown in parenthesis. b, Lymphocytes sensitised with melanoma-1 cells (Δ), BCG-SS (□), or LM-SS (○) and tested against melanoma-1 cells (Δ). Number of immunising tumour cells in parenthesis. When anti-BCG coated melanoma cells and anti-*Listeria* coated melanoma cells were the target cells, results are indicated by broken lines. % Specific lysis of ⁵¹Cr-tumour cells measured in each case.

extracts is related to antigenic similarities, it is conceivable that three tumour cell lines tested may also have common antigenic determinants. This possibility has been tested and the results suggest this is the case (manuscript submitted for publication).

The possibility exists that the cytotoxic reactions observed between the effector and target cells were due to histocompatibility differences. When lymphocytes were tested

as target cells, however, there was considerably less cytotoxicity and sometimes no specific ^{51}Cr release. Lymphocytes from three donors and the breast tumour cells were kindly typed for HL-A antigens by Dr Robert McCalmont, Veterans Hospital, Denver, Colorado. Melanoma-1 was previously typed in Dr Paul I. Terasaki's laboratory, University of California, Los Angeles. No HL-A antigens were found on breast cells and only HL-A 2 was found

Table 1 *In vitro* immunisation of lymphocytes against human tumour cells with bacterial extracts

Lymphocytes from donor no.	Sensitising tumour cell or bacterial extract*	% Specific ^{51}Cr release $\pm$ s.d. from target cells†			
		Breast	Mel-1	Mel-2	Normal lymphoblasts
1	A $\dagger$	Breast	51 $\pm$ 1.0		
		BCG-SS	24 $\pm$ 0.5		5 $\pm$ 2.0
		LM-SS	25 $\pm$ 1.0		6 $\pm$ 1.0
		SA-SS	39 $\pm$ 1.0		10 $\pm$ 2.0
	A $_1$ ‡	Breast	44 $\pm$ 2.0		
		BCG-SS	26 $\pm$ 0.5	23 $\pm$ 1.0	-2 $\pm$ 3.0
2		LM-SS	31 $\pm$ 2.0	37 $\pm$ 1.0	-15 $\pm$ 3.0
		Breast	52 $\pm$ 1.0		
		BCG-SS	15 $\pm$ 1.0		7 $\pm$ 1.0
		SA-SS	29 $\pm$ 2.0		0 $\pm$ 1.0
		EC-SS	4 $\pm$ 0.5		2 $\pm$ 0.5
3		Breast	59 $\pm$ 1.0		
		BCG-SS	32 $\pm$ 1.0	47 $\pm$ 1.0	
		LM-SS	48 $\pm$ 2.0	50 $\pm$ 2.0	
4		Breast	33 $\pm$ 1.0		
		Mel-2		33 $\pm$ 1.0	
		BCG-SS	46 $\pm$ 1.0	38 $\pm$ 1.0	21 $\pm$ 0.5
5		LM-SS	72 $\pm$ 1.0	60 $\pm$ 1.0	25 $\pm$ 1.0
		Breast	62 $\pm$ 3.0		
		BCG-SS	34 $\pm$ 1.0	60 $\pm$ 4.0	
6		LM-SS	17 $\pm$ 1.0	50 $\pm$ 3.0	
		Mel-1		24 $\pm$ 4.0	
7		SA-SS	11 $\pm$ 1.0	9 $\pm$ 1.0	
		Breast	52 $\pm$ 1.0		
8		Mel-1		27 $\pm$ 0.5	
		BCG-SS	55 $\pm$ 1.0	62 $\pm$ 0.5	7 $\pm$ 1.0
		LM-SS	53 $\pm$ 1.0	73 $\pm$ 1.0	-3 $\pm$ 0.5
		Breast	28 $\pm$ 1.0		
9		BCG-SS	16 $\pm$ 0.5	34 $\pm$ 1.0	-1 $\pm$ 0.5
		SA-SS	5 $\pm$ 1.0	20 $\pm$ 1.0	6 $\pm$ 0.5
		EC-SS	5 $\pm$ 0.5	7 $\pm$ 1.0	-3 $\pm$ 1.0
		Breast	9 $\pm$ 0.5	56 $\pm$ 3.0	5 $\pm$ 1.0
10		SA-SS	7 $\pm$ 0.5	15 $\pm$ 1.0	
		EC-SS	7 $\pm$ 1.0	29 $\pm$ 0.5	-1 $\pm$ 1.0
		BCG-SS	5 $\pm$ 1.0	47 $\pm$ 1.0	
11		LM-SS	9 $\pm$ 0.5	54 $\pm$ 1.0	
		SA-SS	3 $\pm$ 0.5	13 $\pm$ 1.0	
		BCG-SS	15 $\pm$ 0.5	78 $\pm$ 0.5	11 $\pm$ 1.0
12		SA-SS	8 $\pm$ 1.0	11 $\pm$ 1.0	8 $\pm$ 1.0
		Breast	15 $\pm$ 0.5		
		BCG-SS	19 $\pm$ 1.0	47 $\pm$ 0.5	5 $\pm$ 1.0
		EC-SS	10 $\pm$ 0.5	17 $\pm$ 2.0	0 $\pm$ 1.0

Lymphocytes were sensitised in 0.75 ml of minimal essential medium containing 20% heat inactivated human AB plasma. Fewer than 1% monocytes were present in sensitised cell population. Cytotoxic activity of sensitised cells was tested against breast, melanoma-1 (mel-1), melanoma-2 (mel-2), and normal lymphoblast cells in ^{51}Cr release assay in triplicate. Normal target cells were normal lymphocytes that have been transformed by PHA or PWM.

*The range of bacterial concentrations used was 18 μg to 36 μg N.

†The adjusted percentage of specific ^{51}Cr release was calculated as:

$$\frac{\text{test release} - \text{minimum release}}{\text{maximum release} - \text{minimum release}} \times 100$$

Controls (minimum values) for each experiment included incubation of target cells alone or target cells with normal unsensitised lymphocytes. Control values were 8–16% for breast, 12–22% for melanoma-1, 18–20% for melanoma-2, and 10–16% for PHA-treated normal lymphoblasts. There was no significant difference between background calculated from target cells incubated alone or target cells incubated with lymphocytes which were cultured alone for 5 d. Maximum values (freeze-thaw $\times 4$) varied between 70 to 83% (average 77%) for melanoma-1, 66 to 72% for melanoma-2, and 68 to 82% (average 72%) for breast tumour cells.

‡A and A $_1$ are two different bleedings from donor 1.

Table 2 Effect of *in vitro* stimulation of lymphocytes with plant mitogens

Lymphocytes from donor no.	Sensitising plant mitogens	% Specific ^{51}Cr release $\pm$ s.d. from target cells			
		Breast	Mel-1	Mel-2	Normal lymphoblasts
1	A† PHA	1 $\pm$ 1.0	36 $\pm$ 3.0		-10 $\pm$ 2.0
	A ₁ † PHA	8 $\pm$ 0.5	61 $\pm$ 1.0	15 $\pm$ 1.0	-2 $\pm$ 1.0
	Con A	-2 $\pm$ 0.5	13 $\pm$ 0.5	8 $\pm$ 0.5	-3 $\pm$ 1.0
2	PHA	1 $\pm$ 1.0	15 $\pm$ 1.0		
	PWM	5 $\pm$ 0.5	42 $\pm$ 1.0		
	Con A	1 $\pm$ 0.5	9 $\pm$ 1.0		
3	PHA	13 $\pm$ 1.0	26 $\pm$ 1.0	10 $\pm$ 2.0	
	PWM	51 $\pm$ 1.0	73 $\pm$ 2.0	49 $\pm$ 1.0	
	Con A	3 $\pm$ 0.5	28 $\pm$ 1.0	24 $\pm$ 0.5	
4	PHA	11 $\pm$ 1.0	50 $\pm$ 0.5	20 $\pm$ 0.5	
5	PHA	0 $\pm$ 1.0	9 $\pm$ 0.5		
	PWM	15 $\pm$ 0.5	35 $\pm$ 1.0		
	Con A	6 $\pm$ 1.0	12 $\pm$ 1.0		
6	PHA	1 $\pm$ 0.5	9 $\pm$ 2.0		
	PWM		-11 $\pm$ 1.0		
	Con A		-12 $\pm$ 1.0		
8	PHA	11 $\pm$ 0.5	24 $\pm$ 0.5		
9	PHA	4 $\pm$ 1.0	21 $\pm$ 1.0		
	PWM	8 $\pm$ 1.0	6 $\pm$ 1.0		
	Con A	-1 $\pm$ 0.5	4 $\pm$ 0.5		
10	PHA	3 $\pm$ 1.0	20 $\pm$ 1.0		0 $\pm$ 1.0

Lymphocytes were sensitised with PHA, PWM, or con A for 5 d and cytotoxic activity was tested against breast, melanoma-1, melanoma-2, and normal lymphoblast cells.

*1:4 dilution of concentration recommended by Wellcome Reagent Ltd. of PHA or PWM was used to stimulate lymphocytes. Con A was used at 1 $\mu\text{g } \mu\text{l}^{-1}$.

†A and A₁ are two different bleedings from donor 1.

on the melanoma-1 cells. Lymphocytes from two of three donors who had HL-A2 were still immunised to melanoma-1 with bacterial extracts. This would suggest that the cytotoxicity observed was not directed towards the histocompatibility antigens of the target cells. The possibility that histocompatibility was not a factor in the phenomenon described will need to be confirmed in a syngeneic system in experimental animals.

In some experiments, cytotoxicity of bacterial immunised lymphocytes was greater than when lymphocytes were sensitised by tumour cells (Fig. 2). In addition, in a series of experiments, ^{51}Cr melanoma-1 cells were incubated with rabbit antisera to BCG or LM for 30 min at 37 °C, washed twice, and then used as target cells. This further augmented the cytotoxicity of bacterial extract immunised lymphocytes (Fig. 2b). Normal sera from the same rabbits that had been obtained before immunisation did not augment cytotoxicity.

Our results indicate that some bacterial antigens can, in *in vitro* conditions, render lymphocytes from normal persons cytotoxic for some tumour cells. The observation that killing of tumour cells by immune lymphocytes was augmented if the tumour targets were coated with antibodies specific for the bacterial extract used suggests in addition that the antibody dependent cellular cytotoxicity (ADCC) mechanism may be involved. ADCC has been shown to be operative against a variety of tumour cells *in vitro*.

The bacterial extracts used in this study are known to consist of many components, only a few of which may be critical for the effects described above. Further studies are needed to isolate and characterise the antigenic portions of bacterial extracts that transform lymphocytes from normal persons into lymphocytes with strong antitumour

activity. It is of interest in this regard that antigenic similarities between melanoma cells and BCG have been demonstrated¹⁷. It is thus tempting to speculate that *in vivo* immunisation with bacteria which crossreacts with tumours may induce cytotoxic lymphocytes to certain tumour cells.

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Cyclic nucleotide fluctuations during steroid induced meiotic maturation of frog oocytes

MATURING oocytes of the frog *Rana pipiens* arrest in prophase of the first meiotic division, when they can be isolated and induced to complete meiotic maturation *in vitro* by exposure to steroid hormone. A study of the meiotic maturation divisions of the frog oocyte therefore encompasses problems of steroid action and the control of cell division. An unusual feature of the interaction of steroids with the oocyte is the independence of the nucleus indicated by the failure of actinomycin D or enucleation to affect the induction of maturation by steroids¹. Furthermore, the interaction is nonspecific, in that several different steroids are capable of inducing maturation *in vitro*². Both cyclic AMP and theophylline inhibit progesterone induced maturation³. This report presents evidence that exposure of prophase-arrested oocytes to progesterone lowers intracellular cyclic AMP and cyclic GMP levels, that theophylline inhibits one component of this response, and that periodic cyclic nucleotide fluctuations occur during the subsequent maturation divisions.

The *R. pipiens* oocyte is particularly useful in an investigation of this nature. *In vitro* maturation of a group of isolated oocytes is intrinsically synchronous, and the appearance of characteristic morphological markers allows their progression through the meiotic divisions to be followed under the dissecting microscope. Furthermore, their extraordinarily large diameter—1,500 μm —allows microinjection of substances to active concentrations without markedly changing intracellular volume.

Gravid *R. pipiens* females obtained from December through April were maintained in artificial hibernation at 4 °C until sacrifice. Oocytes were manually dissected from their ovarian follicles with watchmaker's forceps, and exposed to progesterone at 5 mg l⁻¹ for 10 min. Incubations were carried out in amphibian Ringer's solution (100 mM NaCl, 1.9 mM KCl, 1.1 mM CaCl₂, 1.2 mM NaHCO₃, pH 7.8) at 22 °C. In control groups of 50–100 oocytes, at least 95% underwent proper development as determined by activation of the mature oocyte.

The cyclic nucleotide content of isolated *R. pipiens* oocytes which had not been exposed to progesterone (hereafter referred to as basal level) was 0.6 pmol cyclic AMP (range 0.3–1.1) and 8.0 pmol cyclic GMP (range 5.2–14.9) per oocyte, or approximately 0.6 μmol cyclic AMP and 8.0 μmol cyclic GMP per litre cell water (mean from ten experiments). The mean ratio of basal cyclic GMP content to cyclic AMP content was 15 (range 9–29), in marked contrast to the situation found in the oocytes of other amphibians⁴, and in other cell types (for example ref. 5). Basal levels found in samples of oocytes from the same female showed acceptable variation with standard errors which were 4–5% of the mean (three females, three samples per female, data not shown).

Time courses for fluctuations in the levels of cyclic AMP and cyclic GMP during the progesterone-induced *in vitro* maturation of isolated oocytes are presented in Fig. 1. The data show that significant decreases in the levels of both cyclic AMP and cyclic GMP occur within 30 min after exposure to progesterone. The decreases were not observed in experiments in which the control oocytes did not mature.

An antagonistic interaction between intracellular levels of cyclic AMP and cyclic GMP was formulated by Goldberg in terms of a yin-yang relationship¹¹, and McMahon has applied this concept to the regulation of cell division¹². Curiously, from the time of induction until germinal vesicle breakdown (GVBD), or dissolution of the prophase nucleus, the patterns of cyclic AMP and cyclic GMP

fluctuation are similar, yet after GVBD this trend is reversed and a reciprocal relationship is observed. According to Berridge¹³, these patterns of cyclic nucleotide fluctuations reflect monodirectional and bidirectional

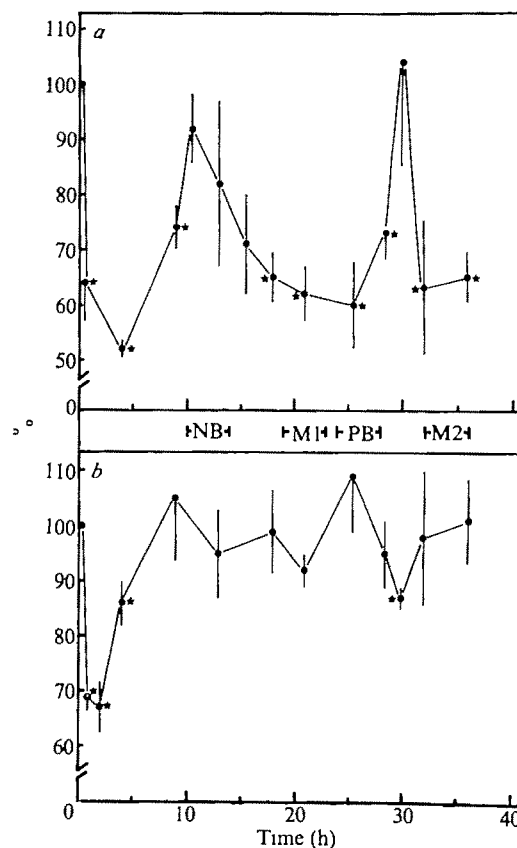


Fig. 1 Cyclic AMP (a) and cyclic GMP (b) levels during progesterone-induced maturation of isolated oocytes expressed as % of basal $\pm$ s.e.m. Abscissa shows time elapsed between exposure to progesterone and sampling. Zero on the time scale refers to basal cyclic nucleotide levels. At the times indicated, either a single sample of 30 oocytes, or duplicate samples of five oocytes were selected under the microscope on the basis of morphology when possible and analysed for cyclic nucleotide content. Cyclic nucleotides were extracted by rapidly homogenising samples of oocytes plus medium in two volumes of absolute ethanol at 0 °C. Immediately following homogenisation 3.8 nCi of ³H-cyclic AMP (38 Ci mol⁻¹) and 2.1 nCi of ³H-cyclic GMP (3.5 Ci mol⁻¹) were added to the homogenate in order to follow recovery from the purification steps. The tritiated cyclic nucleotides amounted to less than 5% and 4% of the total amount of cyclic AMP and cyclic GMP, respectively, in the sample. The homogenate was then centrifuged at 6,000 g for 10 min at 4 °C. The supernatant was flash evaporated under reduced pressure and frozen. Purification and separation of cyclic AMP and cyclic GMP was accomplished by sequential column chromatography over neutral alumina and Dowex 1 $\times$ 8 (formate form) according to Jakobs *et al.*⁶ and Butcher *et al.*⁷, respectively. Cyclic AMP and cyclic GMP were measured by the radioimmunoassay procedure of Steiner *et al.*⁸ as modified by Harper and Brooker⁹. Cyclic AMP was also measured by the protein binding assay of Brown *et al.*¹⁰ Both assay procedures for cyclic AMP gave the same results. Authenticity of cyclic nucleotides was established by incubating aliquots of purified extract with 10 μg of beef heart phosphodiesterase, after which less than 5% of the binding activity in the assays remained. Data from several experiments were pooled by first normalising the data within each experiment, expressing every point as % of basal level in oocytes from that particular female. Values from samples collected less than 90 min apart in different experiments were used to calculate a mean value of several experiments for cyclic nucleotide content at a given time point. All points are mean values $\pm$ s.e.m. from three or more experiments. Student's *t*-test was used to establish by paired comparisons whether there was a statistically significant difference between the mean at a given time point and the basal level (100%). Cyclic GMP was significantly different from basal at only one time point after GVBD. Stars indicate a statistically significant difference from basal ($P < 0.05$ – $P < 0.001$). NB, nuclear breakdown (GVBD); M1, first metaphase; PB, extrusion of first polar body; M2, arrest at second metaphase.

control, respectively. The apparent switchover from monodirectional to bidirectional control suggests that an alteration of cyclic nucleotide metabolism occurs at GVBD.

Berridge proposes that an antagonistic relationship exists between cyclic AMP and Ca^{2+} , with cyclic GMP levels simply reflecting intracellular Ca^{2+} levels. A number of studies have suggested that Ca^{2+} has an important role in the induction of maturation. It has been reported that alterations of calcium flux accompany progesterone induced maturation¹⁴, and that the divalent cation ionophore A23187 (ref. 15), as well as propanolol¹⁶, and lanthanum ions¹⁷ can induce maturation of *X. laevis* oocytes. Ionophore A23187 can induce maturation of *R. pipiens* oocytes (A. B. Kostellow, G. A. Morrill, personal communication), as well as parthogenetically activate ova¹⁸.

Cleavage of the sea urchin egg is accompanied by a cycle of cyclic AMP fluctuations¹⁹ similar to those shown in Fig. 1, and similar fluctuations of cyclic AMP occur during proliferation of mammalian cells in culture²⁰. The significance of these fluctuations has not been established, however.

To investigate the physiological significance of decreased cyclic nucleotide levels in the mechanism of induction of maturation by progesterone, oocytes were exposed to the hormone in the presence of theophylline. Theophylline, a competitive inhibitor of cyclic nucleotide phosphodiesterase, was present at 2.0 mM during a 30 min preincubation, as well as during exposure to hormone, and continuously for the duration of the experiment. At this concentration, theophylline inhibited GVBD and subsequent maturation in 100% of the oocytes in control

groups. Theophylline inhibited the decrease in cyclic AMP levels, but in contrast did not inhibit the decrease in cyclic GMP levels after exposure to progesterone (Fig. 2).

The inhibitory effects of cyclic AMP and theophylline on maturation¹ and of theophylline on the initial decrease in cyclic AMP levels after exposure to progesterone suggest that a decrease in the intracellular levels of this cyclic nucleotide is an early and necessary response of the oocyte in the induction of maturation by the steroid. Without characterising the oocyte adenylate cyclase and cyclic AMP phosphodiesterase(s), the data do not distinguish between activation of a phosphodiesterase and inhibition of adenylate cyclase as the event resulting in the observed decrease in cyclic AMP levels.

The failure of 2.0 mM theophylline to block the decline in cyclic GMP levels after hormone exposure suggests that this cyclic nucleotide is metabolised in a manner different from that of cyclic AMP. Alternatively, the sub-cellular distribution or kinetic properties of cyclic GMP phosphodiesterase different from those of cyclic AMP phosphodiesterase could be responsible for this result.

Cyclic nucleotide fluctuations are thought to influence cellular processes primarily through alterations in the activity of protein kinases^{21,22}. Increases in protein phosphorylation occur at GVBD in both *R. pipiens* and *X. laevis* oocytes^{23,24}. Maller and Krebs²⁵, using micro-injection techniques, have shown that the regulatory subunit of protein kinase mimics progesterone by inducing maturation of *X. laevis* oocytes, while the catalytic subunit inhibits progesterone induced maturation. These results suggest that a decrease in the catalytic activity of oocyte protein kinase is a key step in the induction process in *X. laevis*, and are consistent with a decrease in cyclic AMP levels after exposure of *X. laevis* oocytes to progesterone, similar to that which we report here for *R. pipiens*.

This study of the isolated *R. pipiens* oocyte has revealed a novel mode of steroid action: progesterone causes a reduction in intracellular levels of cyclic AMP that is essential to release of the oocyte from prophase arrest. The significance of very high levels of cyclic GMP relative to cyclic AMP in these cells, and of the later cyclic nucleotide fluctuations have not been determined.

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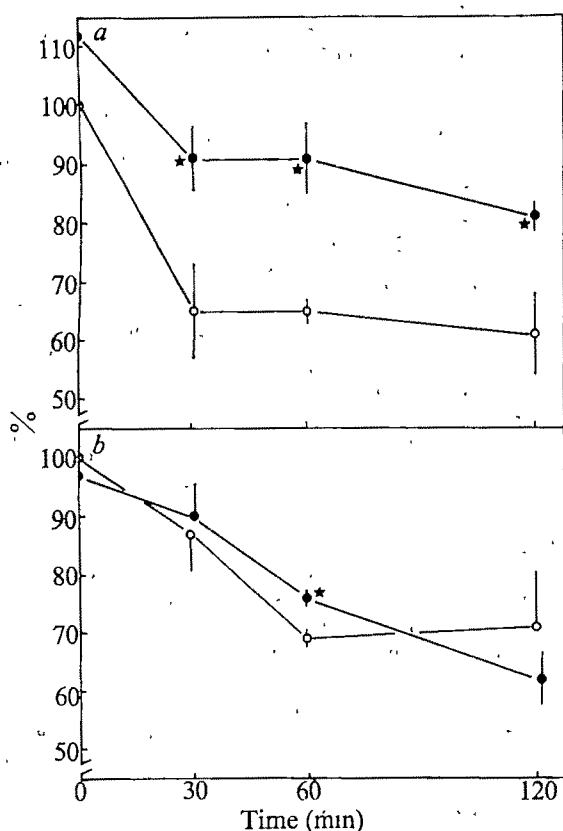


Fig. 2 Effect of theophylline on cyclic nucleotide fluctuations during the induction response. Cyclic nucleotide levels per oocyte expressed as % of basal $\pm$ s.e.m. All points are means from three experiments. Abscissa refers to time elapsed between exposure to progesterone and sampling. Stars indicate a statistically significant difference between control and + theophylline at a given time point ($P < 0.05$ to $P < 0.01$). a, Cyclic AMP levels and b, cyclic GMP levels. ○, Control; ●, plus 2.0 mM theophylline.

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Prostacyclin increases cyclic AMP levels and adenylate cyclase activity in platelets

PROSTACYCLIN (PGX) (ref. 1) is the name proposed for the metabolic product of a recently discovered enzymic transformation of the prostaglandin endoperoxides PGG_2 and PGH_2 (refs 2-4). Prostacyclin synthetase, the enzyme which catalyses this conversion, is particularly active in the microsomal fraction of blood vessel walls^{1,4} although there is evidence that it is also present in the rat stomach fundus². The prostaglandin endoperoxides, PGG_2 and PGH_2 , have been shown to contract arterial smooth muscle and to cause platelet aggregation^{5,6}, whereas, in contrast, prostacyclin relaxes arterial strips and prevents platelets aggregation⁷. A biochemical interaction has therefore been postulated to exist between platelets and the blood vessels wall, such that endoperoxides derived from platelets are converted by the vessel wall into prostacyclin, which in turn actively prevents the deposition of platelets on the vascular endothelium^{2,4}. In general, agents which inhibit platelet aggregation, such as PGE_1 , increase cyclic AMP levels, whereas agents causing aggregation lower platelet cyclic AMP levels⁷. The ability of prostacyclin to rapidly reverse or prevent platelet aggregation suggested that its action on the platelet might, like that of PGE_1 , be mediated by an increase in cyclic AMP levels. This hypothesis is supported by the experiments detailed here which strongly suggest that prostacyclin generated by incubation of arachidonic acid, platelets and aortic tissue, causes a rapid and pronounced accumulation of cyclic AMP by intact platelets, as well as stimulation of adenylate cyclase in isolated platelet membranes.

Human platelet-rich plasma (PRP) was incubated in the presence of theophylline, arachidonic acid, and chopped pieces of pig aorta obtained fresh from a local abattoir. Incubation conditions were similar to those used to generate prostacyclin in incubations of PRP with vascular rings⁸. Cyclic AMP was assayed in samples of incubation media at timed intervals. In the presence of arachidonic acid and aortic pieces, the cyclic AMP content of PRP rose approximately 12-fold within 5 min (Fig. 1a), declining gradually over the next 25 min. In the absence of aortic pieces, arachidonic acid produced no change in platelet cyclic AMP levels. No changes were observed in the cyclic AMP content of platelet-poor plasma (PPP) incubated in the presence of arachidonic acid and aortic rings. When arachidonic acid was omitted from the incubation medium, or the platelet-rich plasma was pre-incubated for 15 min with $100 \mu\text{g ml}^{-1}$ indomethacin, the changes in the cyclic AMP content of PRP were very much diminished, in one case presumably because substrate was absent, and in the other because the inhibition of cyclooxygenase by indomethacin led to reduced prostaglandin endoperoxide levels (Fig. 1b). In some experiments aortic pieces were incubated for 15 min with indomethacin and washed before incubation with PRP and arachidonic acid. This treatment did not alter the effect of aortic tissue on cyclic

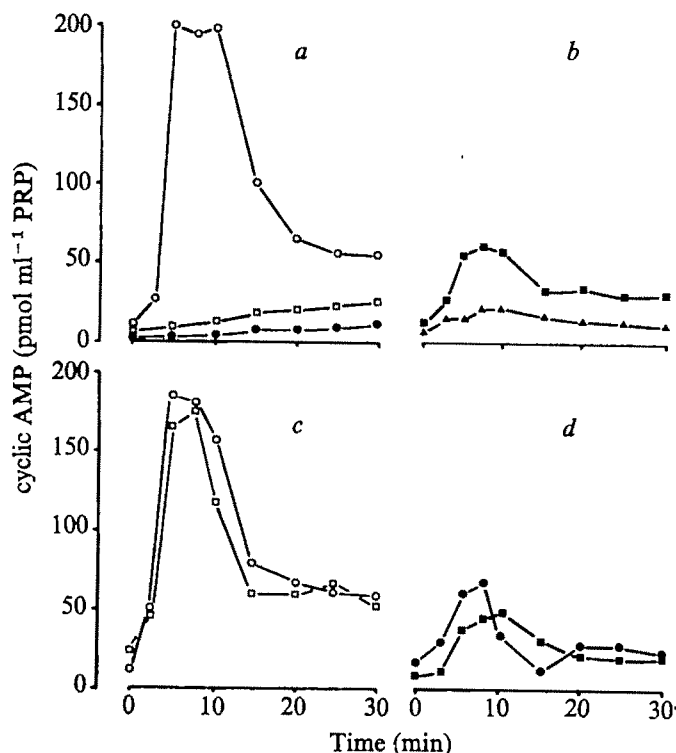


Fig. 1 Cyclic AMP generation by platelets from arachidonic acid in the presence and absence of aortic tissue. PRP was prepared by centrifuging venous blood (9 volumes: 1 volume 3.8% citrate) at 500 g for 10 min at room temperature; PPP was obtained by centrifuging PRP at 5,000 g for 10 min. Reactions were begun by adding 4 ml of PRP or PPP to incubation flasks containing 2 ml buffer (15 mM Tris, pH 7.4, with 120 mM NaCl, 4 mM KCl, 1.6 mM MgSO_4 , 2 mM NaH_2PO_4 , 10 mM glucose and 0.2% bovine serum albumin) and theophylline (10 mM final concentration). Final concentrations of other components of the incubation medium were as follows: fresh pig aortic pieces (50 mg ml^{-1}), arachidonic acid (Sigma; AA, $10 \mu\text{g ml}^{-1}$). Where stated, PRP was pre-incubated for 15 min with indomethacin (I; $10 \mu\text{g ml}^{-1}$). 500 μl samples of incubation media were taken at intervals, boiled for 2 min, and the cyclic AMP content measured using beef adrenal protein kinase competitive binding assay⁹. Standard curves included an appropriate amount of a mixture of buffer and PPP depleted of cyclic AMP by standing overnight at room temperature. Assays were carried out in triplicate on single samples obtained at the indicated time points. The cyclic AMP content of the mixture of buffer and PPP was assayed and the results corrected for this value. a, PRP+AA and aortic pieces ($\circ$); PRP+AA ($\square$); PPP+AA+aortic pieces ($\bullet$). b, PRP+aortic pieces ($\blacksquare$); PRP (+I)+AA+aortic pieces ($\blacktriangle$). c, PRP+AA+aortic pieces+U44069 ($\circ$); PRP+AA+aortic pieces+U46619 ($\square$). d, PRP+aortic pieces+U44069 ($\bullet$); PRP+aortic pieces+U46619 ($\blacksquare$).

AMP levels (results not shown) and is consistent with the reported resistance of prostacyclin synthetase to inhibition by indomethacin. These results are consistent with the view that prostaglandin endoperoxides, intermediates in the metabolism of arachidonic acid by platelets, were converted in the presence of the aortic pieces to a substance which caused an immediate, pronounced rise in platelet cyclic AMP content. The same conditions, used by Bunting *et al.*⁴, resulted in formation of prostacyclin, a potent inhibitor of platelet aggregation.

The prostaglandin endoperoxide analogues (15S)-hydroxy-9 α , 11 α (epoxymethano) prosta 5Z-13E-dienoic acid (U44069) and (15S)-hydroxy-11 α , 9 α (epoxymethano)-prosta 5Z-13E-dienoic acid (U46619) had no influence on prostacyclin formation during the incubation of PRP with aortic rings (Fig. 1c, d), suggesting that as expected, these analogues were not used as substrates by prostacyclin synthetase and neither had any inhibitory effect on the enzyme. But, the addition of an aggregating agent—either

ADP or adrenaline—caused a marked decline in prostacyclin-stimulated platelet cyclic AMP levels (Fig. 2a). This effect was comparable to that of ADP or adrenaline on platelet cyclic AMP levels previously elevated by treatment with prostaglandin E_1 , and the mechanism may be that ADP and adrenaline directly stimulate phosphodiesterase in platelet membranes⁸. The stimulation of cyclic AMP formation during incubation of PRP with arachidonic acid and aortic fragments was prevented by including tranilcypromine (500 μ M) or oxidised arachidonic acid (10 μ g ml^{-1}) (Fig. 2b). Each of these substances has been shown to inhibit the activity of prostacyclin synthetase³.

A sample of incubation mixture of PRP, arachidonic acid and aortic pieces stimulated the activity of adenylate cyclase in partially purified platelet membranes (Fig. 3). Thus, the elevation by prostacyclin of platelet cyclic AMP levels, via the activation of platelet membrane adenylate cyclase, resembles the effects of PGE_1 and may account for the anti-aggregating property of prostacyclin previously demonstrated by Gryglewski *et al.*³. This conclusion receives support now from the demonstration that chemically synthesised prostacyclin is many times more active than PGD_2 and PGE_1 in stimulating platelet production of cyclic AMP and platelet membrane adenylate cyclase^{12,13}.

Involvement of cyclic AMP as an intermediate in prostacyclin action provides a biochemical basis for an important

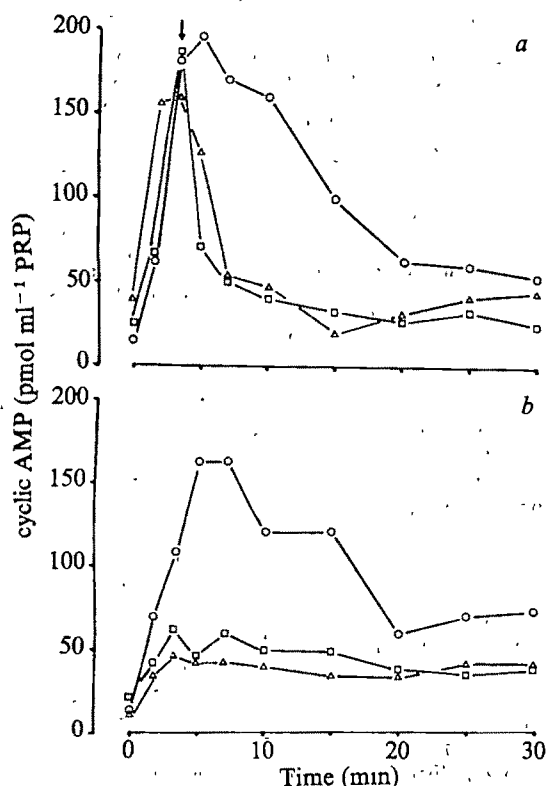


Fig. 2a Effects of ADP and adrenaline on accumulation of cyclic AMP by platelets. Incubations were carried out as described for Fig. 1, and contained PRP in buffer, AA (10 μ g ml^{-1}) and aortic rings (50 mg ml^{-1}). At the time indicated by the arrow, ADP (10 μ g ml^{-1} ; $\square$) or adrenaline (10 μ g ml^{-1} ; $\triangle$) or buffer ($\circ$) was added, and sampling for cyclic AMP assay continued. **b**, Effects of tranilcypromine and of oxidised arachidonic acid on cyclic AMP accumulation by platelets in the presence of aortic pieces. Arachidonic acid was oxidised by dissolving it in buffer at 10 μ g ml^{-1} and exposing it to a stream of air overnight at 25 °C. Incubations were carried out as described in Fig. 1. PRP + aortic pieces + AA ($\circ$); PRP + aortic pieces + AA + tranilcypromine (0.5 mM) ($\square$); PRP + aortic pieces + AA + oxidised arachidonic acid (10 μ g ml^{-1}) ($\triangle$). Assays were carried out in triplicate on single samples obtained at the indicated time points.

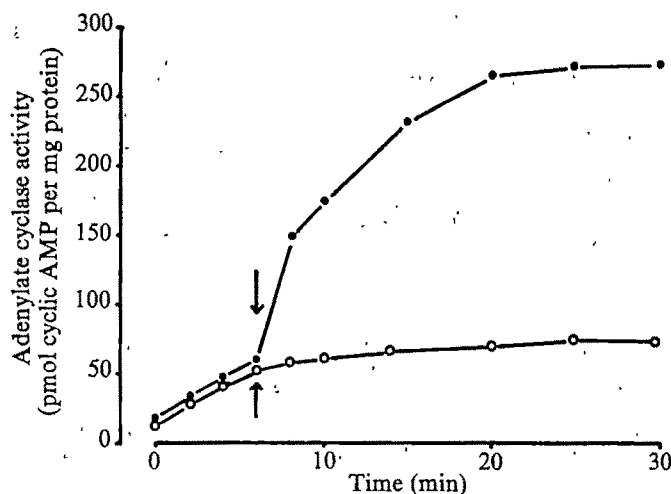


Fig. 3 Adenylate cyclase activity in crude platelet plasma membranes. Platelets were centrifuged from PRP at 5,000 g for 10 min, the platelet pellet disrupted by rapid freezing and thawing (three times) and the crude membrane fraction harvested by centrifuging at 10,000 g . Adenylate cyclase incubations were carried out in 2 ml volumes of 25 mM Tris HCl containing 1 mM ATP³² (10 c.p.m. pmol⁻¹), 4.5 mM $MgCl_2$, 30 mM KCl, 1.5 IU pyruvate kinase; 2 mM phosphoenolpyruvate; 0.013% bovine serum albumin, and platelet membranes. After starting the reactions by addition of platelet membranes, 100- μ l samples were removed at the intervals shown, added to 10 μ l of 40 mM ATP and boiled immediately. The generated ³²P-cyclic AMP was purified by sequential chromatography on Dowex and alumina^{10,11}. At the time indicated by the arrow further additions were made to each flask as follows: $\bullet$, 100 μ l from a 6-ml incubation of PRP, AA (10 μ g ml^{-1}) and aortic rings (50 mg ml^{-1}) which had been incubating for 5 min (see Fig. 1a); $\circ$, 100 μ l from a 6-ml incubation of PPP and AA (10 μ g ml^{-1}) which had been incubating for 5 min (see Fig. 1a).

cellular control system, which produces cooperation between the vascular wall and the blood platelet in order to prevent platelet adhesion to vascular endothelium. In this homeostatic mechanism, the prostacyclin could act continuously upon the platelets to increase cyclic AMP, which in turn may inhibit the platelet cyclooxygenase¹⁴, thereby limiting the amount of endoperoxide available for thromboxane synthesis, and thus turning off production of these potent short-lived stimulators of platelet aggregation. If prostacyclin or related compounds are found to be produced in other tissues, studies of their effects on target tissues will be greatly facilitated if they are found to have the general property of stimulating adenylate cyclase activity.

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Calcium-dependent somatostatin secretion from rat neurohypophysis *in vitro*

THE tetradecapeptide somatostatin originally identified in hypothalamic extracts, has been subsequently shown by bioassay, radioimmunoassay and immunohistochemical techniques to be extensively distributed throughout the central nervous system, in the gastrointestinal tract, and in the pancreas¹⁻⁵. Within the central nervous system, immunoreactive somatostatin has been localised to fibres in the external zone of the median eminence, to neuronal cell bodies in the anterior hypothalamus, to axons in the posterior pituitary lobe, as well as in spinal ganglia and in fibres in the dorsal zone of the spinal cord⁶. Although strong indirect evidence exists for the release of somatostatin from the hypothalamus into the portal vessels in physiological conditions⁷ there are no direct studies of somatostatin secretion from nerve cells. Since the neurohypophysis contains a high concentration of somatostatin⁸, and can be readily isolated, we used it as a model for studying the *in vitro* release of the peptide from nerve terminals. We report that secretion is markedly increased following potassium stimulation, provided calcium is present in the incubation medium.

Male Sprague-Dawley rats (240-300 g) were killed by decapitation, the posterior pituitary lobes were isolated, separated from adhering intermediate lobe tissues and incubated in pools of five in 1 ml bicarbonate-buffered Locke's solution at 37 °C under an atmosphere of 95% O₂-5% CO₂ with constant shaking. After a 30 min preincubation period, the medium was replaced by fresh Locke's solution or a modified Locke's solution at 30 min intervals. Medium from each incubation period was collected in glass tubes at 0 °C. On completion of each experiment the neural lobes were extracted by sonication in 1 ml 1-M acetic acid. Media and extracts were measured for somatostatin by a specific radioimmunoassay⁹ with a mean sensitivity of 1.4 pg. The protein content of the extracts was determined by the method of Lowry *et al.*¹⁰.

A high concentration of immunoreactive somatostatin was found in the posterior pituitary extracts. This contrasts with the absence of somatostatin in the anterior pituitary⁸. The total neurohypophysial content of somatostatin was determined by adding the amount of immunoreactivity released in the incubation media to the content remaining in the neurohypophysial lobes at the end of the experiment and yielded a value of 773±64 pg per neural lobe (mean±s.e.m. of 84 pools of five lobes each) or a concentration of 4.9±0.4 pg per µg protein. Somatostatin was secreted at a low basal rate from the isolated neural lobes, the amount released 5.9±1.4 pg per neural lobe per 30 min, representing 1.4±0.35% of the total content (mean of 20 experiments, Fig. 1).

High extracellular potassium is a potent stimulus for neurohypophysial hormone secretion and this response can be potentiated by a lowering of the extracellular sodium ion concentration¹¹. The release of somatostatin was also markedly increased following potassium stimulation to 14.8% of total content per 30 min ($P<0.01$, Fig. 1a). This stimulatory effect was calcium dependent and was abolished

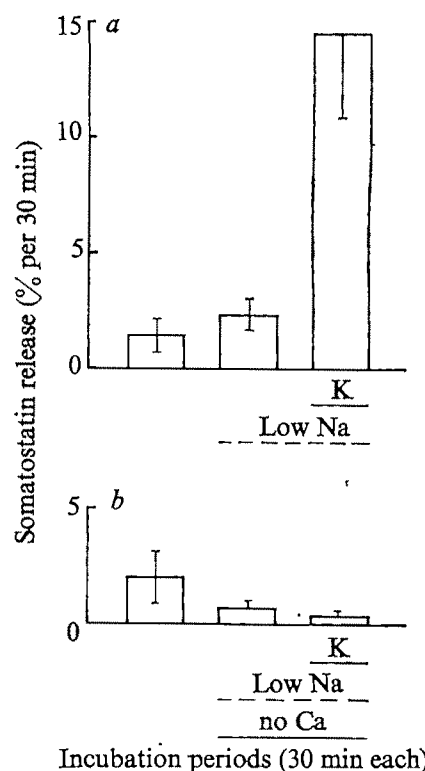


Fig. 1 Somatostatin-like immunoreactivity released by pools of five rat neural lobes during successive 30 min incubation periods. Each column represents the mean ± s.e.m. of 10 experiments. The initial incubation medium (first column in a and b) contained (in mM): Na⁺, 160; K⁺, 5.6; Ca²⁺, 2.2; Mg²⁺, 1.0; Cl⁻, 160; HCO₃⁻, 12 and glucose, 10. In a, the Na⁺-concentration of incubation medium was lowered to 12 mM for the second and third incubation periods; in addition, the K⁺-concentration was raised to 56 mM during the third period. Isoosmolarity was maintained by adding appropriate amounts of choline. In b, the same protocol was followed except that Ca²⁺ was replaced by Mn²⁺ (2.2 mM) during the second and third incubations. Values are expressed in % of total content of neurohypophysial somatostatin released per incubation period.

when extracellular Ca²⁺ was replaced by an equimolar concentration of Mn²⁺ an antagonist of Ca²⁺ in secretory processes (Fig. 1b, $P<0.01$).

The present studies confirm our earlier observation of the presence of immunoreactive somatostatin in the rat neurohypophysis⁸. Somatostatin thus represents a further addition to the list of peptides found in the posterior pituitary which includes oxytocin, vasopressin and thyrotrophin releasing hormone¹¹. Although the posterior pituitary concentration of somatostatin is comparable with that of other somatostatin rich neural tissues (for example, cerebral cortex, brain stem, spinal cord⁶) it is over 1,000 times lower than the concentration of the classical neurohypophysial hormones, oxytocin and vasopressin^{12,13}.

The mechanism of somatostatin release from the posterior pituitary shares two important characteristics with the neurosecretory process: stimulation of release in response to membrane depolarisation and dependence of this process on extracellular calcium. The functional significance of neurohypophysial somatostatin remains to be determined. One possibility is that somatostatin stimulates the release of vasopressin as suggested by *in vitro* studies¹⁴. Second, since somatostatin released in the posterior pituitary is capable of reaching the anterior pituitary through the short portal vessels, it seems likely that neurohypophysial somatostatin along with somatostatin released from the median eminence may have a role in the regulation of growth hormone and thyroid stimulating hormone secretion.

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Brain thyrotrophin-releasing hormone is independent of the hypothalamus

THYROTROPHIN-releasing hormone (TRH) is widely distributed in regions of the brain outside the hypothalamus¹⁻³, and behavioural⁴ and neurophysiological⁵ studies lend credence to the hypothesis^{1,6} that in these locations it is involved in neurotransmission. To determine whether extrahypothalamic brain TRH arises from the hypothalamus or is synthesised *in situ*, we have made electrolytic lesions of the "thyrotrophic area"⁷ of the rat hypothalamus, and examined the effect on TRH content in the hypothalamus itself, the rest of the brain and the anterior and posterior pituitary. We have found that such lesions markedly reduce the hypothalamic content of TRH, deplete the pituitary gland of TRH and result in severe chemical hypothyroidism, but do not affect the large amount of TRH normally stored in the extrahypothalamic brain.

We placed bilateral electrolytic hypothalamic lesions with a platinum electrode (Stoelting lesion maker) (3mA for 30 s) in male Sprague-Dawley rats (Charles River) under ether anaesthesia. The coordinates⁸ chosen—AP 6.4 mm, lateral 0.7 mm on each side of midline, vertical +1.5 mm—were designed to produce a large lesion in the region of the paraventricular nucleus extending towards, but not involving, the median eminence or pituitary stalk. Such lesions produce marked impairment of pituitary-thyroid function⁹. Six out of nine animals survived the procedure. In the seven control rats bilateral electrolytic lesions were placed in the cerebral cortex 2 mm below the surface. Both groups weighed about 400 g.

The effectiveness of the procedure was assessed 1 week later by determination of serum thyroxine (T₄) by immunoassay on blood removed by orbital puncture from the rats under ether anaesthesia. T₄ levels in the lesioned rats were all lower than the values in the controls (mean $\pm$ s.e.m.) 0.7 ± 0.1 compared with 4.1 ± 0.4 $\mu\text{g dl}^{-1}$ ($P < 0.001$).

To obviate the effect of hypothyroidism *per se* on tissue TRH content, all animals (test and control) were given orally sodium-L-thyroxine ($12 \mu\text{g d}^{-1}$, a dose designed to provide normal thyroid replacement) in 20 ml of a synthetic liquid diet (Sustacal) for 14 d, the last dose of thyroxine being given 48 h before termination of the experiment. The animals were decapitated and the brains were removed immediately. The hypothalamic lesions were confirmed visually with the aid of a dissecting

microscope, and hypothalami from both groups were excised and placed in 2 ml of 90% methanol for extraction; the anterior and posterior pituitaries were treated similarly. Tissue taken for the hypothalamic fragment was bounded anteriorly by the optic chiasm, posteriorly by the anterior margin of the mammillary nucleus, laterally by the hypothalamic sulcus and dorsally by a plane connecting the anterior commissure and anterior margin of the mammillary nucleus. To extract TRH from the brain, it was extruded through a 21-gauge needle into 10 ml of 90% methanol. The dried methanol extracts of all the tissues were reconstituted in phospho-saline buffer and assayed for TRH by a specific radioimmunoassay as before¹.

Although the hypothalamic content of TRH was much reduced in the lesioned rats compared with controls ($3,625 \pm 249$ compared with $9,357 \pm 449$ pg; $P < 0.001$), the amount of TRH in the extrahypothalamic brain ($17,040 \pm 925$ compared with $18,929 \pm 737$ pg) was similar in both groups; indeed the concentration of TRH was identical in the lesioned and normal animals (70 pg per mg protein¹⁰). In contrast the TRH content was markedly lower in the anterior pituitary (15 ± 8 compared with 64 ± 5 pg; $P < 0.001$) and posterior pituitary (< 2 compared with 157 ± 32 pg; $P < 0.001$) (Table 1). The lower TRH in anterior pituitary likely resulted because less TRH was delivered to the pituitary.

Table 1 Effect of a lesion of the 'thyrotrophic area' of the hypothalamus on brain distribution of TRH in the rat

	Lesion	Control	Significance
Hypothalamus	$3,625 \pm 249$	$9,357 \pm 449$	$P < 0.001$
Anterior pituitary	15 ± 8	64 ± 5	$P < 0.001$
Posterior pituitary	< 2	157 ± 32	$P < 0.001$
Extrahypothalamic brain	$17,040 \pm 925$	$18,929 \pm 737$	$P > 0.1$

Results (mean $\pm$ s.e.m.) are given as pg per tissue. Six lesioned and seven control animals were studied.

This study shows that a lesion of the hypothalamus resulting in reduced hypothalamic content of TRH and impaired TRH secretion from the hypothalamus, as implied from the low circulating levels of thyroxine, does not affect extrahypothalamic brain TRH content, suggesting that the latter is synthesised *in situ* and is independent of the hypothalamic secretion. Complementary studies were done by Brownstein *et al.*¹¹ who showed that after hypothalamic deafferentation the extrahypothalamic brain TRH content was unaltered whereas the hypothalamic TRH content was reduced, raising the possibility that much of the hypothalamic TRH is synthesised by cells outside this region.

The high concentration of TRH in the posterior pituitary relative to the anterior pituitary in the normal group (560 ± 9 compared with 28 ± 2 pg per mg protein; $P < 0.001$) has been observed before^{1,3}. The depletion of TRH from the posterior pituitary in the lesioned animals supports the concept of a third neurosecretory hypothalamo-hypophyseal system extending into the neurohypophysis, as suggested by Hökfelt *et al.*¹² on the basis of immunohistochemical staining of TRH¹³ and somatostatin¹⁴ positive fibres reaching into the posterior pituitary from the median eminence. Whether TRH has a role in posterior pituitary function is uncertain, but it may be relevant that TRH is found in high concentrations in the pituitary complex of lower vertebrates¹, and there is evidence (reviewed by Sawyer¹⁴) that in the bony fish the neurohypophysis may be an homologue of the median eminence in higher animals.

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Synaptic responses mediated by identified histamine-containing neurones

EFFORTS to prove that histamine has a role in synaptic neurotransmission have been less successful than for other biogenic amines. For example, there has been no convincing physiological or pharmacological evidence to support the transmitter candidacy of this imidazole at any known synapse. In the central nervous system (CNS) of the marine mollusc *Aplysia californica* chemical measurements of single isolated neurones have revealed large concentrations of histamine within two identified neurones¹. The histamine-containing neurones (HN) uniquely possess a specific histidine decarboxylating enzyme and they can synthesise and store labelled histamine². These biochemical observations prompted the suggestion³ that the two identified nerve cells utilise histamine as a neurotransmitter. The physiological studies reported here show that each HN monosynaptically elicits multicomponent excitatory and inhibitory postsynaptic potentials (p.s.ps) in different follower neurones, the somal membranes of which show appropriate potential changes to iontophoretic application of histamine. This suggests that histamine is a neurotransmitter released from the nerve terminals of the HN.

In the absence of ultrastructural evidence needed to prove the monosynaptic nature of cell-to-cell connectivity, several physiological criteria provide a reliable basis on

which to distinguish between direct and indirect synaptic connections (reviewed in ref. 3). These criteria include: (1) that the postsynaptic response follows the presynaptic action potential in a one-for-one manner and that there is a constant latency of response; (2) that the postsynaptic responses persist in medium containing increased concentrations of Ca^{2+} , and (3) that synaptic efficacy increases after intracellular injection of tetraethylammonium (TEA) into the presynaptic neurone. These tests have been applied in the work reported here to support the monosynaptic character of synaptic responses elicited by the HN.

Desheathed cerebral ganglia dissected from *Aplysia* (200-350 g) were perfused with artificial seawater (ASW, 492 mM, NaCl; 10 mM, KCl; 20 mM, MgCl_2 ; 30 mM, MgSO_4 ; 60 mM, CaCl_2 ; 10 mM, Tris). The pH of the ASW was adjusted to 7.8 and the temperature was maintained at 18 °C by a Peltier device. Standard electrophysiological techniques were used for intracellular stimulation and recording with double-barrelled glass microelectrodes filled with 2 M potassium citrate or 3 M TEA bromide. In several experiments the identity of the HN was biochemically confirmed after intracellular recording from pre- and post-synaptic neurones by assaying the activity of histidine decarboxylase, which is a cell-specific marker for HN in *Aplysia*⁴.

In either normal or Ca^{2+} -enriched ASW solutions the HN are silent, displaying little spontaneous synaptic and action potential activity. Several identifiable neurones located on the dorsal surface of the ganglion showed consistent synaptic effects after action potentials evoked in the HN. These follower neurones are symmetrically located on both the left and right sides of the ganglion: they show (1) identical responses to ipsilateral HN stimulation, (2) similar spontaneous synaptic and action potential rhythms and (3) antidromic responses to electrical stimulation of the corresponding ipsilateral and contralateral cerebral nerve trunks. No synaptic responses could be observed between left and right HN nor between ipsilateral HN and contralateral follower neurones; intracellular dye (ccalt) injection also indicates that neither the HN nor the follower neurones send axons across the midline of the ganglion (my unpublished observations).

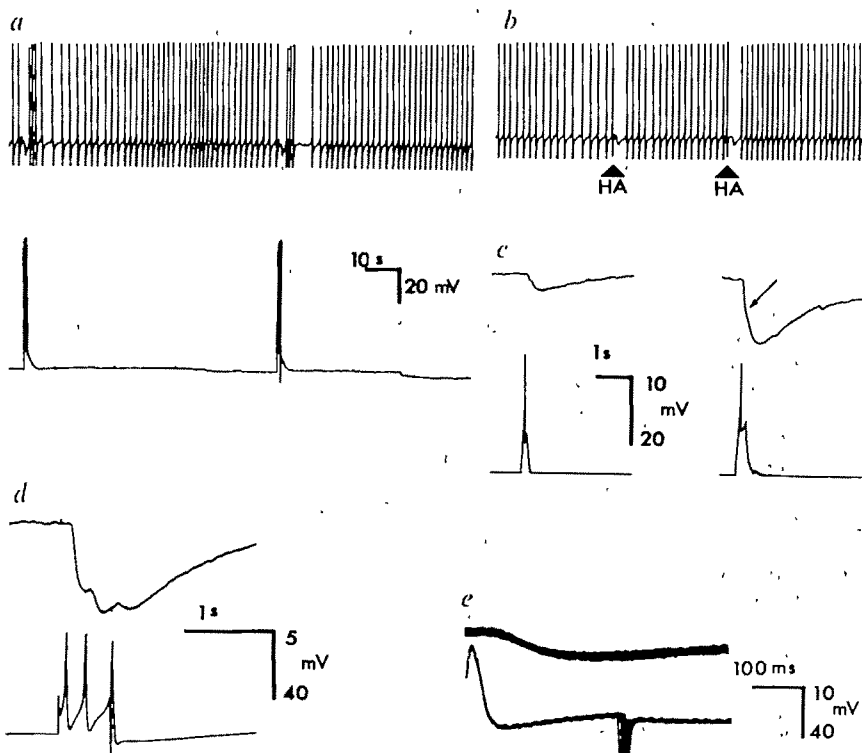


Fig. 1 Inhibitory effects produced by histamine-containing neurone (HN) spikes on one class of follower neurones. *a*, Inhibition of spontaneous spike activity in a follower neurone evoked by HN spikes. *b*, Inhibitory response to iontophoretic application (30 nA, 0.5 s) of histamine (HA) delivered to the somal membrane of the same follower cell; action potential amplitudes smaller than in (*a*) due to frequency response characteristics of the recording channel. *c*, i.p.s.ps recorded after spontaneous spiking was prevented by pre-setting the membrane potential to -55 mV. Right trace shows i.p.s.p. 5 min after TEA was injected into the HN by passing current between two intracellular TEA electrodes. Inflection point on the rising phase (arrow) of the i.p.s.p. depicts the fast phase of the two-component i.p.s.p. *d* and *e*, Fast i.p.s.ps recorded in a similar follower neurone from another cerebral ganglion at 4 °C to abolish the slow i.p.s.p.; membrane potential preset to about -50 mV. *d*, Association between HN spikes and fast i.p.s.ps; *e*, six superimposed records of fast i.p.s.ps and HN spikes illustrating constant latency of the evoked i.p.s.p.; latency of fast i.p.s.p. exaggerated by cooling. In this and subsequent figures upper voltage calibrations are associated with upper traces (follower neurones); lower voltage calibration corresponds with lower traces (HN).

Figure 1a shows the effects produced by a train of HN spikes on the endogenous activity of one class of follower neurones. At the normal resting potential, about -50 mV, as few as five HN spikes rapidly depress spontaneous spiking activity in these neurones. Thus the influence of the HN on these follower cells is clearly inhibitory. Spiking activity in the follower neurones was also inhibited by iontophoretic application of histamine (Fig. 1b), suggesting that histamine receptors are present on these postsynaptic membranes. When the membrane potential of the follower neurone was hyperpolarised beyond spiking threshold, hyperpolarising p.s.ps (i.p.s.ps) were observed. These are composed of two components, an initial rapid i.p.s.p. with a fast rise time and short duration (200–400 ms) and a slow i.p.s.p. with a gradual rise time and a prolonged decay phase (several seconds). The amplitude of the fast i.p.s.p. is usually small at membrane potentials near -60 mV and frequently obscured by the slow i.p.s.p. The fast i.p.s.p. becomes evident, however, when transmitter release from the HN is potentiated by intracellular administration of TEA or by cooling the preparation below 7°C , which reversibly suppresses the slow i.p.s.p. Figure 1c shows the i.p.s.ps before and 5 min after injection of TEA when the duration of the presynaptic action potential had more than doubled and the i.p.s.ps were substantially increased in duration and amplitude. The one-to-one nature of the fast i.p.s.p. and its constant latency at 4°C are illustrated in Fig. 1d and e, respectively. In other experiments where the slow i.p.s.p. was also eliminated by cooling, it was possible to reverse the polarity of the fast i.p.s.ps at membrane potentials greater than -65 mV (E_{Cl}) (ref. 4). Thus, the sensitivity to cooling of the slow i.p.s.ps, a known characteristic

of potassium-dependent potentials in *Aplysia*⁴, and the polarity change of the fast i.p.s.p. below -65 mV suggest that these fast and slow i.p.s.ps are mediated by chloride and potassium conductances, respectively.

Another class of postsynaptic response is illustrated in Fig. 2. Endogenous spiking activity in these follower neurones was inhibited by HN stimulation and by the application of histamine. Although these followers are functionally inhibited by spike activity in the HN, a small excitatory component always precedes the hyperpolarising response. This early component is more apparent when the membrane potential of the follower neurone is polarised to prevent spiking. The sample records shown in Fig. 2 b–d illustrate the postsynaptic responses at greater amplification and at different membrane potentials. When the membrane potential was increased, the excitatory component (arrows) became more prominent and at -80 mV the response was almost entirely excitatory. Similarly, the hyperpolarising response to exogenously applied histamine decreased and at -80 mV the histamine response was biphasic. Thus it seems that two types of histamine receptors may exist on the membranes of these follower neurones, one mediating a hyperpolarising response and the other associated with depolarisation. The biphasic nature of the p.s.ps is further illustrated in Fig. 2e–h when the membrane potential of the follower neurone was preset to -60 and -90 mV. The synaptic responses shown reveal several characteristics of these biphasic responses: (1) they are composed of a rapid (200–400 ms) depolarising component (e.p.s.p.) and a slowly developing and prolonged (s) i.p.s.p.; (2) the equilibrium potential of the i.p.s.p. is near -80 mV because it was reversed in polarity at -90 mV

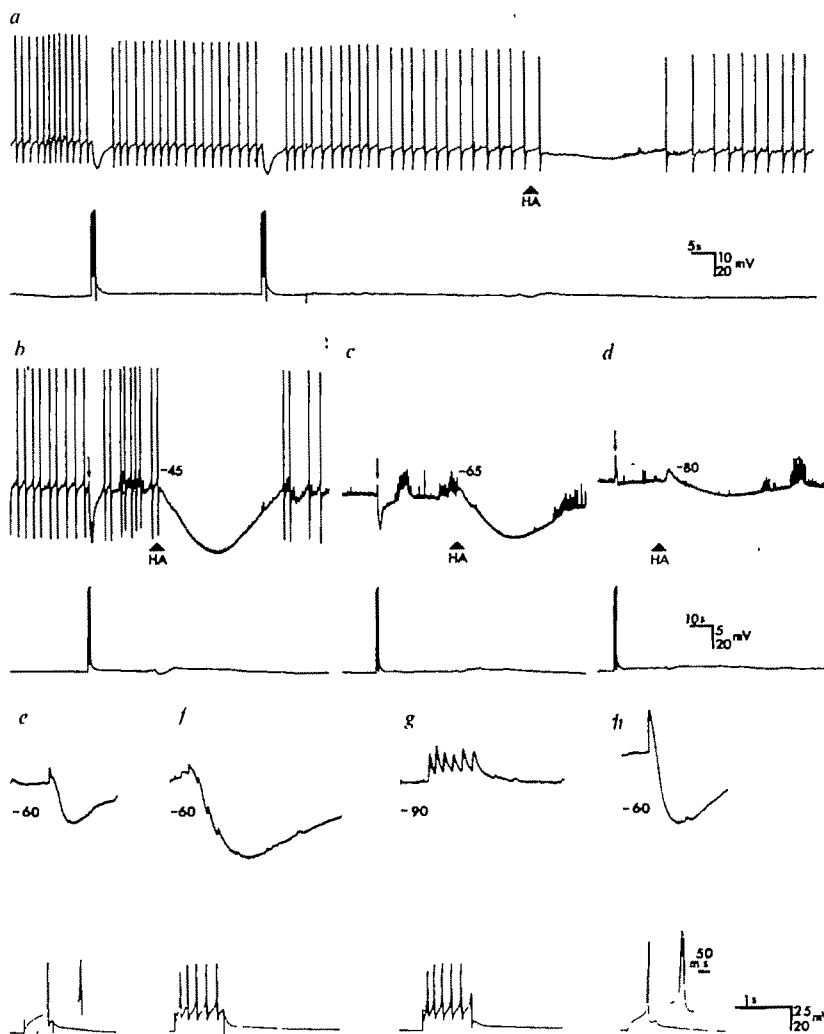


Fig. 2 Biphasic responses in another class of follower neurones. a, Effects of trains of HN spikes and the application of histamine (HA) on spontaneous spiking activity of the follower neurone recorded at resting potential (-45 mV), histamine applied by diffusion from a micropipette. b–d, Effects of HN spikes and histamine recorded at various membrane potentials shown in each set; arrows indicate the onset of the early excitatory synaptic component of the evoked response. e–g, Biphasic p.s.ps recorded at different membrane potentials and after various numbers of HN spikes. h, Biphasic p.s.p. 30 min after TEA was intracellularly injected into the HN; insets near lower traces show duration of HN spikes before (e) and 30 min after TEA administration (h). All records from the same follower neurone.

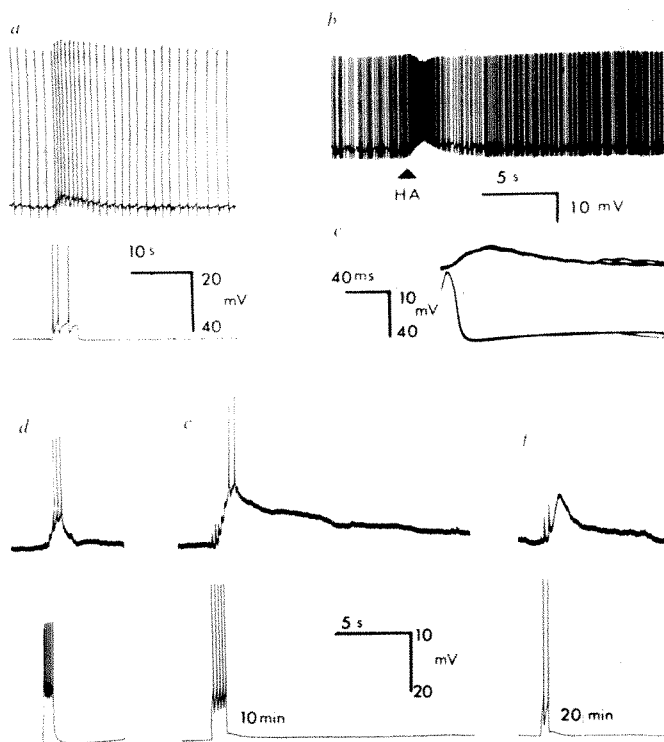


Fig. 3 Excitatory responses elicited by HN stimulation. *a*, Effects of three HN spikes on the endogenous activity of a follower neurone. *b*, Excitatory responses to iontophoretic application (20 nA, 0.5 s) of histamine (HA) delivered to the somal membrane of another follower neurone receiving excitatory synaptic input from HN. *c*, Four superimposed traces of e.p.s.ps and HN spikes recorded from the same follower as illustrated in (*b*). *d-f*, Effects produced by intracellular TEA into the HN in another preparation bathed in ASW containing normal (10 mM) Ca^{2+} ; membrane potential preset to -55 mV. *d*, Control responses; *e* and *f*, obtained at times indicated after administration of TEA. At 20 min after injection, two HN spikes produce fast and slow e.p.s.ps, with amplitude and duration substantially larger than control response to a train of seven HN spikes. E.p.s.ps in *d* and *e* cause firing of the follower neurone.

(Fig. 2g) and nearly absent at -80 mV (Fig. 2d); and (3) the amplitude of the i.p.s.ps, unlike those of the e.p.s.ps, were potentiated by repetitive firing of the HN (compare traces *f* and *g* in Fig. 2). Figure 2h shows the increased HN spike duration and the accompanying increases in the amplitudes and durations of both e.p.s.p. and i.p.s.p. components of the biphasic p.s.p. 30 min after TEA injection.

In a third class of follower neurones, stimulation of the HN produced excitatory responses, that is an increase in the rate of spiking (Fig. 3a); these followers also show an excitatory response to iontophoretically applied histamine (Fig. 3c). Like the two previously described synaptic responses, these excitatory followers also received two-component p.s.ps—a fast depolarising component (fast e.p.s.p.) several hundred milliseconds in duration and a long-lasting (seconds) depolarising component (slow e.p.s.p.). The sample records shown in Fig. 3c illustrate the constant latency between the HN spikes and the fast e.p.s.p. The two-component nature of these excitatory synaptic responses is clearly observed when transmitter release is enhanced by prolonging the HN spike by injection of TEA or by lowering the temperature of the bathing medium (data not shown). The responses shown in Fig. 3d–f reveal the effects of intracellular injection of TEA on the e.p.s.ps recorded from another preparation bathed in normal ASW (10 mM Ca^{2+}). Ten minutes after injection the amplitude and duration of both fast and slow e.p.s.ps were substantially increased and by 20 min the responses were even further potentiated.

These results demonstrate that single HN excite some follower neurones and inhibit others, and that the synaptic potentials underlying these effects are multicomponent, consisting of fast and slow p.s.ps in various combinations: fast i.p.s.p.+slow i.p.s.p.; fast e.p.s.p.+slow i.p.s.p.; and fast e.p.s.p.+slow e.p.s.p. Multiphasic p.s.ps elicited by single presynaptic neurones are not an uncommon feature of molluscan synapses^{6–11}, and multi-receptor systems for proven or suspected neurotransmitters have been characterised on molluscan neurones^{12–15}. The tests used to distinguish between polysynaptic and monosynaptic connections indicate that all synaptic responses evoked by HN are mediated by monosynaptic pathways. Even though the measurements of latency and constancy of the slow synaptic responses are compromised by their gradual onset and long duration, the persistence of the slow p.s.ps. in high- Ca^{2+} ASW and their enhancement produced by intracellular injection of TEA is consistent with the monosynaptic nature of these slow responses.

The observations that neurones receiving monosynaptic connections from the HN respond to exogenously applied histamine in a manner qualitatively similar to the effects produced by HN stimulation demonstrates that histamine could act as a neurotransmitter at these identified synapses. This view is strengthened by the observations that histamine-sensitive neurones are but a small fraction of the neurones within this nervous system (ref. 16 and my unpublished results). Thus from the results described here together with previous biochemical data^{1,2} showing that these same presynaptic neurones uniquely synthesise and contain substantial amounts of histamine, it seems that this system of identifiable nerve cells will be useful for clarifying whether histamine is actually released as a synaptic neurotransmitter.

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Reduced β -adrenergic receptor concentrations in ageing man

CONCENTRATIONS of certain hormone receptors are known to be reduced during ageing in several animal species^{1–4}. Most relevant work, however, has been limited to intracellular hormone receptors such as those for steroids, and less is known about changes in cell surface receptors, including those for catecholamines. We have measured the concentration of β -adrenergic receptors in crude membrane fractions of mononuclear cells from human subjects of various ages. Receptor affinities for ($-$) ^3H -dihydroalprenolol were essentially the same for all subjects. But a

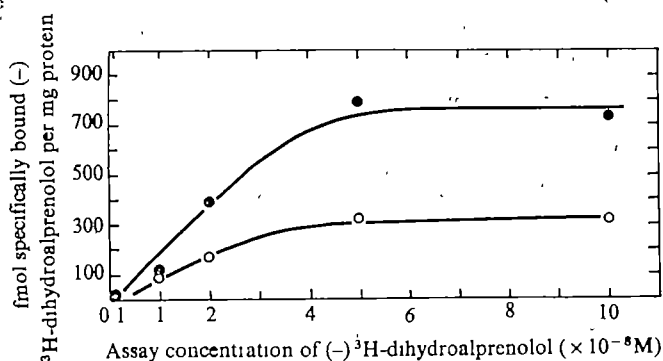
significant decrease in receptor concentration was observed in cells from older individuals.

Subjects were 23 males between 24 and 81 yr old, without significant illnesses and taking no medications. Some were paid volunteers and others were recruited from the longitudinal study of ageing of the National Institute on Ageing and thus were living in a community and fully ambulatory. The study lasted 6 months with a random distribution of subjects by age. We made multiple determinations of receptor concentrations on several subjects during 2–3 months. Subjects fasted and did not smoke for 12 h before the study. With written consent, 112–225 ml of blood was withdrawn by antecubital venipuncture into heparinised syringes, with subjects in the supine position. Whole blood was subjected to density gradient centrifugation by the method of Böyum, using a Ficoll-diatrizoate mixture (specific gravity 1.077)⁵. Mononuclear cell fractions thus obtained were assayed for β -adrenergic receptors by a modification of the method of Williams *et al.*⁶. This method uses the labelled β -adrenergic antagonist (–)³H-dihydroalprenolol incubated at various concentrations (10^{-8} to 10^{-7} M) with washed crude membrane preparations in the presence and absence of 10^{-3} M unlabelled (–) dihydroalprenolol to determine specific binding. Numbers of lymphocytes were determined by morphological criteria using Turks solution and the nonspecific esterase staining technique adapted from Yam *et al.*⁷. Cell viability was evaluated by Trypan blue exclusion. Protein determinations were made by the method of Lowry *et al.*⁸.

The specific binding of (–)³H-dihydroalprenolol to the washed crude membrane preparation was saturable, of high affinity and stereospecific in favour of the (–) stereoisomer. These findings are consistent with those of Williams *et al.*, who showed that binding detected by this method possesses the characteristics of the β -adrenergic receptor⁶. Lymphocytes constituted 83% of the mononuclear cells obtained. Viability as assessed by Trypan blue exclusion was generally greater than 94%. Cell yield, cell viability and protein yield per cell did not vary with the age of the donor. Saturation plots of β -adrenergic receptor concentrations in crude mononuclear membrane preparations of two representative subjects are shown in Fig. 1. At saturation levels (greater than 5×10^{-8} M (–)³H-dihydroalprenolol) specific binding accounted for approximately 50% of total binding, although at lower concentrations the percentage of specific binding was greater, as reported by Williams *et al.*⁶.

The receptor has high affinity for (–)³H-dihydroalprenolol in preparations from both young and old subjects, with K_{DS} of approximately 20 nM for each group. The mean saturation level of young subjects (24–41 yr, $n = 11$), however, was 572 ± 58 pmol mg⁻¹ protein ($\pm$ s.e.m.) while for subjects older than 46 yr ($n = 12$) the corresponding value was slightly greater than half that value,

Fig. 1 Specific binding of (–)³H-dihydroalprenolol to crude mononuclear cell membranes of representative subjects. ●, 24-yr-old; ○, 75-yr-old.



332 ± 48 pmol mg⁻¹ protein ($\pm$ s.e.m. $P < 0.01$). When all data are considered (Fig. 2), there is a significant negative correlation of receptor concentration with age ($r = 0.641$, $P < 0.001$).

Receptor number per cell also correlated inversely with age from approximately 14,000 to 8,000 sites per cell in the young and old groups, respectively. Receptor levels in individual subjects remained fairly constant over short periods (2–3 months).

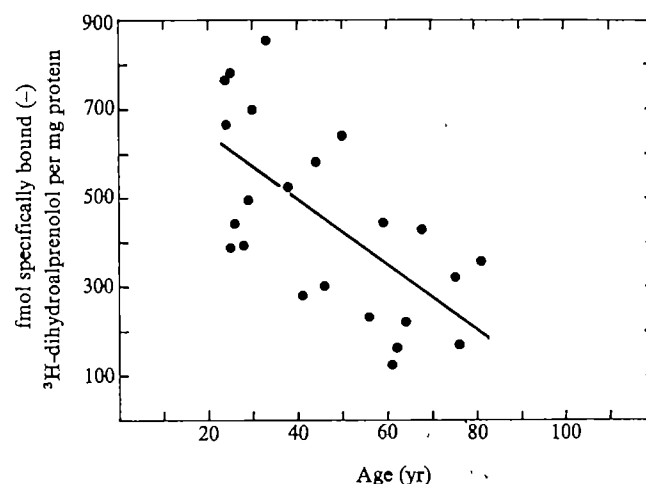


Fig. 2 Maximal specific binding of (–)³H-dihydroalprenolol to crude mononuclear cell membranes of subjects aged 24–81 yr.

The search for physiological and biochemical determinants of ageing has led to investigations of age-associated alterations in hormone receptors. Steroid receptor concentrations are known to decline with age in various rodent tissues^{1,2,4,9,11}, and the ability of steroid hormones to elicit physiological responses has been shown to be diminished with age in several of these tissues^{1,10,11}. Whereas steroid hormone receptors are intracellular, some receptors located on the cell surface also seem to change with age. Concentrations of rat hepatic insulin receptors and adipocyte glucagon receptors, however, seem to decline very early in life^{12,13}.

Previous work with human material has dealt with either intracellular receptors or cells in tissue culture. Binding of glucocorticoids to intracellular receptors in human liver obtained postmortem is diminished in older individuals³. The effect of age on binding of insulin to cultured human fibroblasts has also been studied, but the results have been controversial^{14,15}. Our investigation is the first to demonstrate age-associated alterations of surface hormone receptors in cells taken directly from man.

The aetiology of these receptor alterations remains unclear. Differences in the composition of mononuclear cell populations or individual changes in the mononuclear cells might result in variation between young and old individuals. Relative and absolute numbers of T and B lymphocytes have been thought to remain fairly stable during human ageing^{16,17}, but this has been questioned⁸. Selective removal of monocytes from the mononuclear cell preparation by a nylon mesh column does not markedly influence the amount of (–)³H-dihydroalprenolol specifically bound⁶. The values obtained for the concentration of β -adrenergic receptors of young (age 24–41 yr) subjects, while higher than those initially published⁶, compares more closely with values currently obtained using similar methods (ranging from 96 to 476 fmol (–)³H-dihydroalprenolol specifically bound per mg protein) (personal communication from K. Kariman and R. J. Lefkowitz). Additionally, diminished concentrations of β -adrenergic receptors have been reported in frog erythrocyte membranes previously

exposed to catecholamines *in vivo*¹⁹. This catecholamine-induced 'desensitisation' is another possible aetiology for receptor decline in ageing man, where plasma catecholamine levels are known to increase²⁰.

Altered responsiveness to catecholamines has been observed during ageing in several rodent and human organ systems as well as in intact man²¹⁻²⁴. Several immunological functions of lymphocytes are known to be influenced by adrenergic agents²⁵. The possible role of age-associated receptor reduction or 'desensitisation' in altering the effects of catecholamines on these functions requires elucidation.

Our findings complement previous studies in several aspects. Our results lend further support to the idea that loss of certain hormone receptors is a frequent manifestation of ageing. The demonstration of diminished β -adrenergic receptor concentrations in ageing man offers a possible explanation for the observed age-associated loss of adrenergic responsiveness.

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Tuning properties of cochlear hair cells

THE mammalian cochlea is generally considered to be a frequency analyser. When the ear is stimulated by a pure tone the basilar membrane vibrates. The amplitude of the vibrations varies along the length of the membrane with the position of the maximum depending in a graded way upon the frequency of the tone^{1,2}. The basal turn responds maximally to high frequencies, the apex to low frequencies. Consequently hair cells situated along the membrane are frequency selective. The nerve fibres innervating the basilar membrane have low thresholds over a narrow range of frequencies corresponding to the displacement maxima of the section of the basilar membrane that they innervate³. But the neural tuning curves (the relationship between threshold and frequency for individual nerve fibres) are much sharper than the mechanical tuning curves of the basilar membrane (the relationship between amplitude of displacement and frequency at a single point on the membrane) and this discrepancy has led to the proposal of a "second filter"⁴. In this paper we describe the tuning of intracellularly recorded receptor potentials from histologically identified hair cells (IHC) of the guinea pig cochlea

in an effort to investigate the nature of the hypothetical sharpening process and we conclude that IHCs are tuned as sharply as auditory nerve fibres. To our knowledge this is the first time intracellular recordings have been made from inner hair cells in the mammalian cochlea.

Guinea pigs weighing 120-200 g were maintained throughout the experiment under pentobarbitone-phenoperidine anaesthesia and were paralysed with Flaxedil (gallamine triethiodide) and artificially ventilated (method of E. F. Evans, personal communication). The cochlea was exposed ventrolaterally through the acoustic bulla. A fine Teflon insulated silver wire was positioned close to the round window and secured to the skull with cyanoacrylic cement so that the threshold (within 10 dB) of the compound action potential (AP) evoked by pure tone bursts of 2 ms duration could be monitored during the exposure of the basilar membrane. The sound source was a driven half-inch condenser microphone (B&K 4133) in a hollow ear bar which was inserted in the tympanic ring and secured to the temporal bone and adjacent bones with cyanoacrylic and dental cement. The basilar membrane of the basal turn was exposed taking care to minimise bleeding into the scala tympani thus preserving AP threshold. All results presented in this paper are uncorrected for the sound system which is measured to be flat within 10 dB. The sound pressure levels (SPL) indicated are referred to 0 dB SPL re 2×10^{-5} N m⁻².

Microelectrodes were drawn from 1 mm o.d. fibre filled boro-silicate glass tubing on a Livingstone type electrode puller. They had resistance of about 700 M Ω when filled with 6% procion yellow, but this was reduced to around 300-400 M Ω by bevelling the electrode tips (on a grinding wheel) to facilitate penetration of the basilar membrane⁵. Despite the reduction in their resistance by bevelling, the frequency response of the electrodes was too low to record cochlear microphonics and we have therefore concentrated on measuring the d.c. receptor potential (summing potential). The electrodes were advanced under visual control into the basilar membrane with a hydraulic micro-

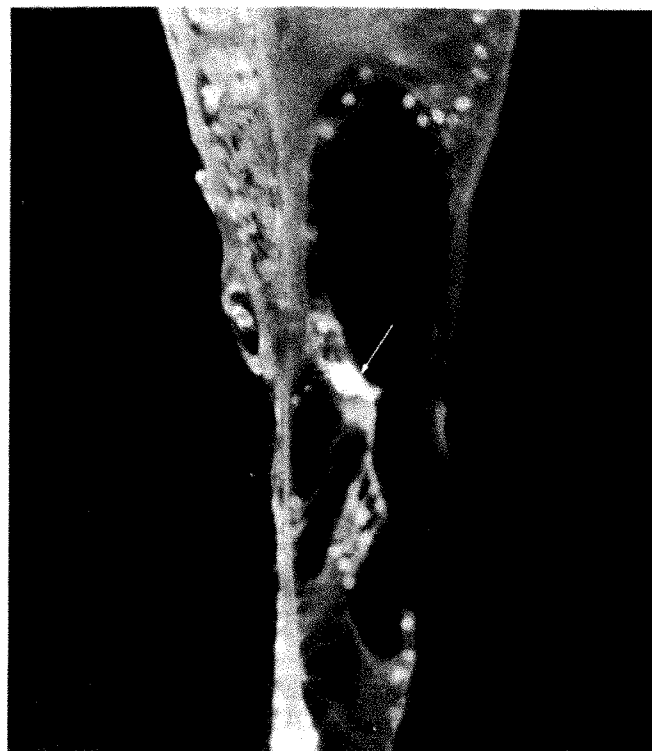


Fig. 1 An inner hair cell (arrow) filled with Procion Yellow in the basal turn of the right cochlea of animal 128. The photomicrograph was taken with ultraviolet illumination and the scale bar indicates 25 μ m.

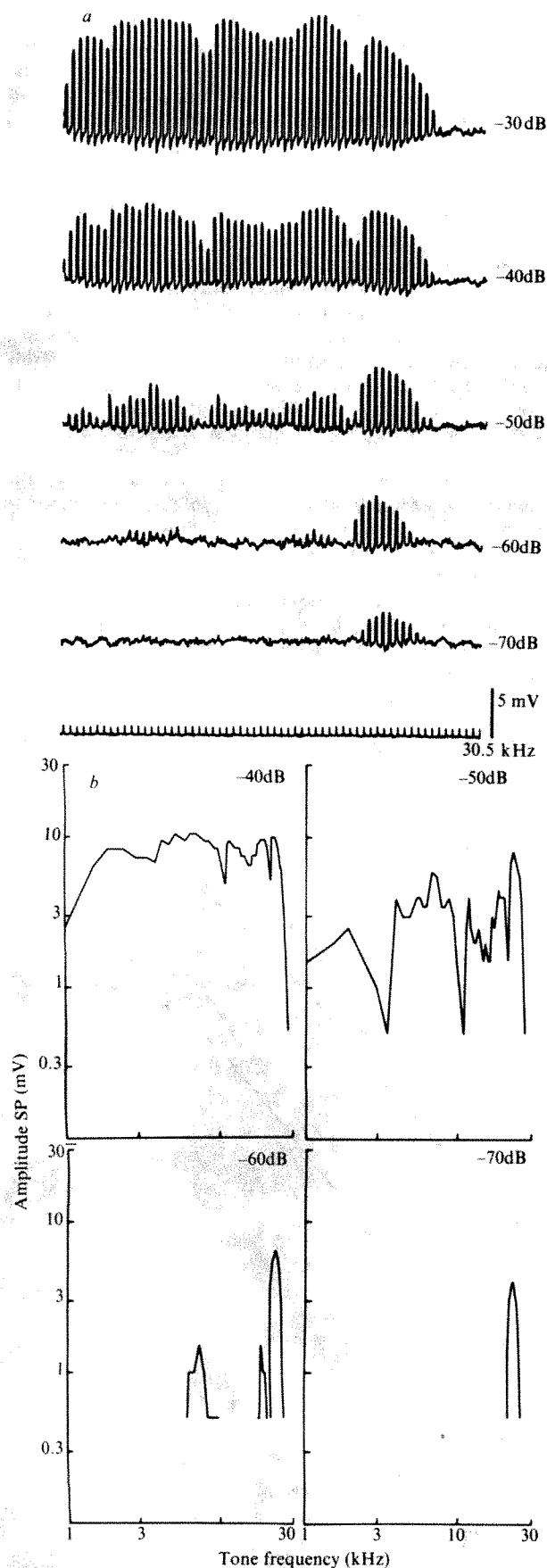


Fig. 2 *a*, Receptor potentials recorded intracellularly from an identified inner hair cell in the basal turn of the right cochlea of animal 105 in response to 15-ms tone bursts (rise and fall time 2 ms) presented at 470-Hz intervals. Trigger pulses for the tone bursts are shown in the lower trace beginning at 1 kHz and ending at 30.5 kHz. *b*, Isointensity curves of the receptor potentials shown in (*a*) plotted at their different SPL.

drive and were accurately positioned when the perilymph was momentarily drained. On rare occasions there was a transient rise in threshold after draining the scala tympani, however sensitivity always returned as the scala tympani refilled.⁶

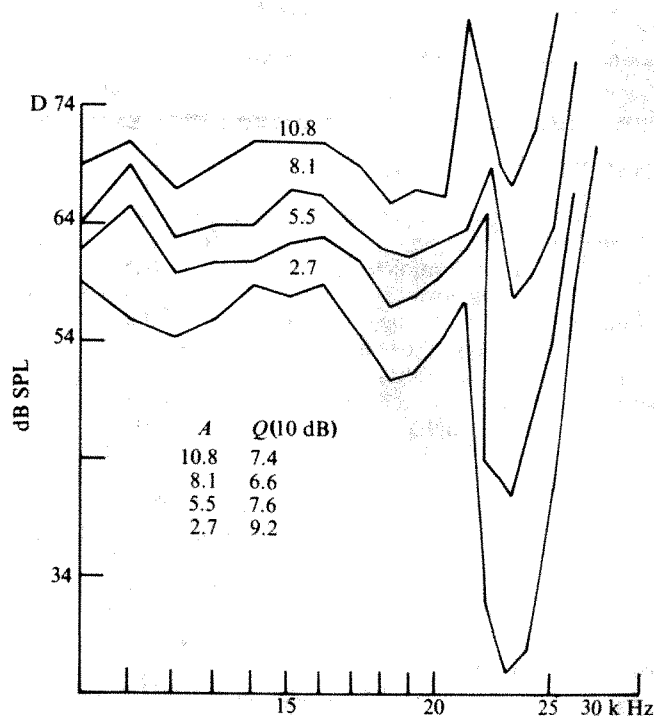
The first two or three cells encountered by the micro-electrode in the region of the IHCs had resting potentials of 80–90 mV and small depolarising potentials of 0–1 mV at 74 dB SPL at characteristic frequency (CF). When the electrode was advanced further, cells were encountered with resting potentials between 35–45 mV and with positive receptor potentials between 5 and 17 mV at 74 dB SPL at CF; negative receptor potentials were never recorded intracellularly.

Membrane potentials were sometimes stable for 10 min or more, but on occasion dropped within a minute of penetration. The magnitude of the receptor potential seemed to vary with the amount of damage sustained by HCs during penetration and was not highly correlated with the AP threshold. Of 26 dye marked cells with large receptor potentials, nine were successfully found under the microscope and were identified as IHCs. An example is shown in Fig. 1. Only the data from these cells are discussed here.

The frequency response of the IHCs was determined between 1 and 40 kHz by presenting 15-ms tone bursts at 470-Hz intervals. At high SPL (74 dB), the frequency response is similar to that of the basilar membrane. However, as the SPL is reduced the receptor potentials at frequencies below the CF fall faster than those at the CF so that at 44 dB SPL the cell is very sharply tuned. Figure 2 illustrates the response of a cell with a CF of 23 kHz.

The interpretation of our results rests largely on the relative importance of the d.c. receptor potential (summing potential) and cochlear microphonic in exciting the afferent nerve fibres. At AP threshold the receptor potential can be as large as 1.6 mV and increases with the level of stimulation to a maximum of about 17 mV, 70 dB above threshold. These recorded potentials are larger than receptor potentials recorded in hair cells in other organs^{7,8} and compare in magnitude with presynaptic potentials recorded elsewhere in the nervous systems⁹. Thus, in view

Fig. 3 Isoamplitude curves of intracellular receptor potentials from experiment 105. *A*, Amplitude of receptor potential in mV.



of their size, it may be that the d.c. receptor potential is responsible for exciting the auditory nerve. If this is so, then the tuning properties of the auditory nerve depend upon the tuning properties of the receptor potential.

To determine whether the frequency response of the intracellular receptor potential corresponds to that of auditory nerve fibres, the receptor potential data must be converted into isoamplitude curves since neural tuning curves are plots of threshold against frequency (isorate). Isoamplitude plots of receptor potential against frequency converted into isoamplitude curves since neural tuning curves are level dependent and, therefore, a comparison with neural tuning curves must be made at a receptor potential that corresponds to the neural threshold. Since AP threshold (which corresponds to single fibre threshold) was 10 dB below the lowest SPL at which receptor potentials were measured, the lowest isoamplitude plot (2.7 mV) is considered to be the closest to the neural threshold tuning curve.

The sharpness of auditory nerve tuning curves is measured in terms of the Q_{10dB} (CF divided by the bandwidth 10 dB above the threshold at CF) and this reaches a maximum of about 10 in the most sensitive fibres³. The Q_{10dB} of the 2.7 mV isoamplitude curve is 9.2 and we therefore conclude that intracellularly recorded receptor potential is tuned as sharply as the auditory nerve and hence that any sharpening of the frequency response beyond that of the basilar membrane mechanics does not involve neural interaction.

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Lysogeny by f2 phage?

Two prominent properties of lysogenic bacteria are, first, inheritable potentiality for phage production and, second, immunity against homologous phage. Both characteristics are unaffected by growth in the presence of phage anti-serum or by serial subcultures of single colonies. This indicates that every lysogenic cell carries the genetic information for the biosynthesis of a given type of the phage particle¹; so far, however, this has been known only for some DNA bacteriophages. I present here the first demonstration that bacteria can also contain the genetic information for the production of an RNA phage. If *Escherichia coli* bacteria are grown and infected with f2 RNA phage in liquid medium supplemented with guanidinium chloride, many cells subsequently behave as true lysogens. They are 'immune' to f2 and spontaneously release phage identical in morphology, plaque type, serological property, male specificity and single-stranded RNA genome, to the phage used for the initial infection.

f2 is a very small virulent phage and is male specific—

adsorbing only to F pili of male *E. coli* strains. Its single-stranded RNA chromosome has two functions: it is the infectious part of the phage particle, and acts as viral messenger in the infected cell that controls the synthesis of two structural proteins and RNA replicase². This dual function for viral single-stranded RNA is also found in animal poliovirus. Guanidine prevents the synthesis of viral RNA by guanidine-sensitive strains of poliovirus by inhibiting completely the replication of viral genome³. Whether guanidinium chloride has a similar effect in bacteria, that is, inhibition of replication of f2 RNA, is not known.

Guanidinium chloride at a final concentration of 10 mM in Tryptone liquid medium⁴, does not interfere with growth and colony forming ability of *E. coli* bacteria. But when bacteria grown in the presence of guanidinium chloride were infected by f2 in otherwise optimal conditions for f2 growth⁴ the yield of phage was strongly reduced and the percentage of bacteria surviving was markedly increased (Table 1).

To distinguish between resistance and immunity, the guanidinium chloride-treated and f2 infected (multiplicity of infection < 1) male bacteria were allowed to grow with aeration for 3 h after the infection and plated on G-agar (agar⁴ supplemented with 10 mM guanidinium chloride) with anti-phage serum. The colonies thus obtained were picked out, and, when plated on G-agar with the indicator bacteria in the top agar⁴, some of them (about 16%) gave rise to plaques. It is possible that the serum did not inactivate all the phage attached to the F pili. To check this, aliquots of f2-producing bacteria were inoculated into liquid G-medium supplemented with anti-phage serum (at the concentration that inactivated more than 99% of the

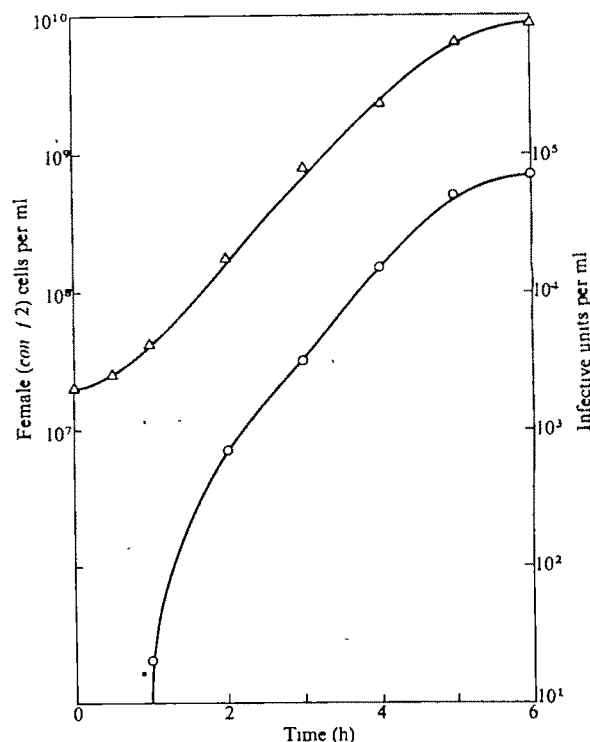


Fig. 1 Spontaneous release of the phage by *E. coli*(con. f2) cells. F⁻(con. f2) bacteria were grown overnight in G-medium supplemented with anti-phage serum at the concentration that inactivates more than 99% phage during 5 min. After two washings by centrifugation in saline-calcium and treatment with phage antiserum⁴, the bacteria were inoculated into fresh G-medium (zero time) and incubated with aeration up to the stationary phase of growth. The number of viable bacteria (Δ) and of infective units (○) were estimated hourly (as described in Table 1). Essentially the same results were obtained with male(con. f2) bacteria.

Table 1 Immunity of (*con. f2*) bacteria to f2 phage

Bacterial culture	³ H-leucine-labelled phage ³ H- sedimented with		³ H-uridine-labelled phage ³ H- sedimented with		f2 yield per ml of infected culture	Bacteria surviving infection (%)
	Antiserum (%)	Bacteria (%)	Antiserum (%)	Bacteria (%)		
Hfr RA-2	86	5	14	77	7.8×10^{11}	26
Hfr RA-2, G-medium	82	8	11	75	6.1×10^9	84
Hfr RA-2(<i>con. f2</i>)	84	7	13	78	1.3×10^6	91

To label phage RNA, ³H-uridine (specific activity 7.4 Ci mmol⁻¹, Radiochemical Centre, Amersham) was added to bacterial culture 30 min before infection, at 2.5 µCi ml⁻¹; to label phage coat, ³H-leucine (150 mCi mmol⁻¹, Radiochemical Centre) was added to bacterial culture 30 min before infection, at 2.5 µCi ml⁻¹. Phage stocks were prepared¹ and purified by density gradient centrifugation in CsCl. The phage labelled in its RNA or protein were used separately, at a multiplicity of 0.8, to infect *E. coli* Hfr RA-2 (from Dr K. B. Low), growing either in Tryptone medium or in Tryptone medium supplemented with 10 mM guanidinium chloride (G-medium), and *E. coli* Hfr RA-2(*con. f2*) growing in G-medium at 37 °C (using experimental procedure for f2 growth¹). 10 min after the infection, free phage (that is, phage still attached to F pili, phage ghosts and truly free phage) were removed by violent agitation in a Towers shaker for 3 min. Thereafter the infected bacteria were freed from free phage by two washings of the cells by centrifugation in saline-calcium⁴ and sedimented by centrifugation at 3000 r.p.m. for 5 min (Sorval centrifuge, rotor SS34). Free phage (in the supernatant) were treated with f2 antiserum for 10 min at 37 °C and sedimented at 10,000 r.p.m. for 30 min. The pellet of infected cells was resuspended into fresh medium (Tryptone or G-medium) and that of free phage into saline-calcium. One aliquot of the bacterial suspension was used for the estimation of radioactivity⁵ (in Table 1: ³H sedimented with bacteria), and other for the examination of bacterial survival⁶ and phage production by one cycle of growth¹, using Hfr RA-2 as an indicator strain. Free phage were used for the estimation of radioactivity⁵ (in Table 1: ³H sedimented with antiserum). The results represent average values from three separate experiments. Essentially the same results were obtained with other male bacteria: *E. coli* F⁺CR 63 from Dr M. Radman, and *E. coli* F⁺ts lac⁺ pro⁺/lac⁻ pro⁻ from Dr D. J. Cummings (the latter grown at 30 °C). f2 phage was donated by Dr A. D. Kaiser.

phage during 5 min), grown to stationary phase and then plated again with antiserum. After five successive re-isolations (after long growth with anti-phage serum in each case) bacteria showing completely new characteristics were isolated: (1) they were not resistant to f2, but did not produce the phage when infected, as though they had been 'immune' to f2 (Table 1) and (2), during the growth in the liquid medium they released phage (Fig. 1). These bacteria are designated (*con. f2*), that is, containing the genetic information for f2 biosynthesis.

It is unlikely that anti-phage serum treatment did not remove all the phage and that some phage still remained

adsorbed to F pili of male bacteria. If this were to occur, however, it would not explain the 'immunity' observed. Definite proof that the phenomenon described here is not due to f2 particles adsorbed to F pili was obtained by constructing F⁻(*con. f2*) bacteria using F⁺ts (temperature sensitive) cells for infection. At the inhibitory temperature, the thermosensitive F⁺ plasmid is diluted out from the *E. coli* population, because its replication is inhibited and it is inherited unilinearly by the descendants of the original F⁺ bacteria⁷. One aliquot of *E. coli* F⁺ts lac⁺ pro⁺/lac⁻ pro⁻ cells was allowed to grow in G-medium at 42 °C, while the other was infected in the same medium at 30 °C with f2, and after 10 min warmed to 42 °C by adding fresh medium prewarmed at 60 °C. The growth of cells was continued at 42 °C for 3 h, and infected and non-infected bacteria were plated at 42 °C on MacConkey agar—enabling us to distinguish male from female bacteria⁸. Colonies of female cells were picked out and F⁻(*con. f2*) bacteria were made as above for (*con. f2*) male cells; the only difference was that bacteria were grown at 42 °C. Controls on MacConkey agar were used at each step of F⁻ and F⁻(*con. f2*) strain isolation. The female cells thus obtained were lac⁻ pro⁻, and they could not adsorb male-specific phage f2, R17 and MS2. F⁻(*con. f2*) bacteria, however, produce phage spontaneously (Fig. 1). This phage is, by its morphology (Fig. 2), male specificity, serological property, single-stranded RNA genome (examined by the method of Bradley⁹) and plaque type (on Tryptone agar), identical to the phage f2. But on G-Tryptone agar mottled plaques occur, from the turbid zone of which male (*con. f2*) bacteria can be isolated. The functional state and the location of genetic information for phage production in (*con. f2*) bacteria, and the molecular mechanism of guanidinium chloride action are under investigation.

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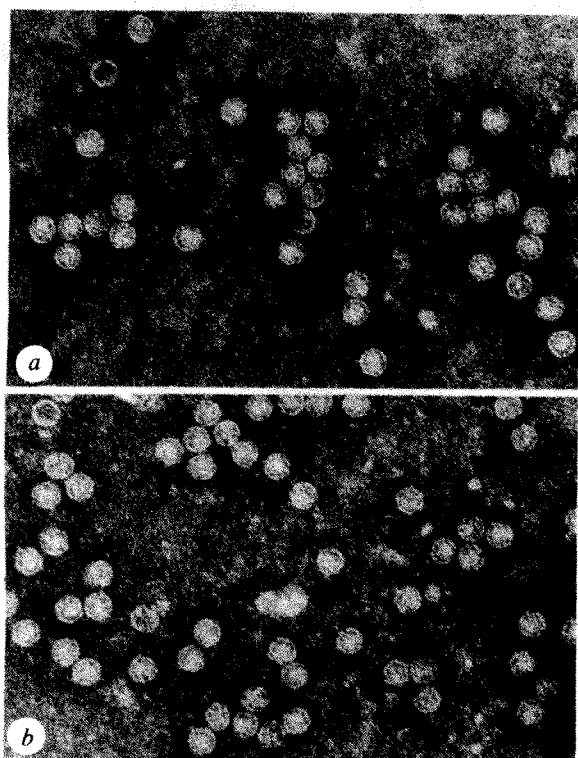


Fig. 2 Electron micrographs showing the morphology of phage. Phage, prepared by negative staining, were examined in a Siemens Elmiscop I. a, Wild type f2 particle; b, phage released spontaneously by F⁻(*con. f2*) cells. (×150,000.)

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Ether induced morphological alteration of Pf-1 filamentous phage

BACTERIOPHAGES of filamentous structure have been isolated in several species of bacteria and all have similar physico-chemical properties¹. They consist of single-stranded circular DNA and two species of proteins—A protein and B protein. They are released from infected cells without disintegrating the cells and are inactivated on exposure to chloroform although the presence of lipids in the virion has not been reported. During a study of Pf-1, a filamentous phage which propagated on strain K of *Pseudomonas aeruginosa*², we found that it is almost completely inactivated with ether, whereas phage fd and Xf are refractory to it. Ether sensitivity of the viruses is generally thought to be an indication of the presence of lipids in the virion. Pf-1 might, therefore, be thought to contain lipids in its structure. We report here a study of the inactivation and morphological alteration of Pf-1 induced by ether and the results of chemical analysis of Pf-1 which suggest the absence of lipids in this virus.

For the propagation of Pf-1 and fd, nutrient broth was used and Xf was propagated in the medium described by Kuo *et al.*³. The effects of organic solvents on the plaque forming activity of filamentous phages are shown in Table 1. Pf-1 was completely inactivated by ether and other solvents tested, whereas fd and Xf were only inactivated with chloroform.

Table 1 Inactivation of filamentous phages by organic solvents

Treatment	Pf-1	Titre (PFU ml ⁻¹)		
		Xf	fd	
None	1.3×10^{11}	1.6×10^{11}	4.2×10^{11}	
Ethyl ether	$< 10^3$	1.5×10^{11}	3.6×10^{11}	
Chloroform	$< 10^3$	8.0×10^7	1.5×10^7	
Methanol	$< 10^3$	NT*	4.2×10^{11}	
Acetone	$< 10^3$	NT	3.0×10^{11}	

Culture fluid containing about 10^{11} PFU ml⁻¹ phage was mixed with an equal volume of solvent. The mixture was stirred gently with a pipette and allowed to stand for 10 min at room temperature. Phage activity in the aqueous phase of the mixture separated by low-speed centrifugation (500g, 5 min) was titrated using a plaque technique.

*NT, Not tested.

Electron microscopic observation of ether treated Pf-1 showed that phage filaments disappeared and many rod-like structure appeared (Fig. 1a). This morphological change observed in Pf-1 could be the reason for the loss of the activity of this phage. The width of the rod was 185 Å and the length was variable but always shorter than that of the phage filament. The middle portion of the rod was densely stained with phosphotungstate. From these electron microscopic images two types of structure for the rod can be postulated. One is a hairpin like structure which can be formed by joining two phage filaments bent like a hairpin, and the other is a hollow cylinder formed by the swelling of a phage filament. Shadowing techniques revealed the surfaces of the rod-like structures, and the grooves which would be expected from the images of hairpin like structures were not observed (Fig. 1b). The effects of ether on the activity of Pf-1 were rapid, and after exposure to the ether vapour for even a few seconds a decrease of its activity could be measured. Electron microscopic observation of this preparation showed that phage filaments were swelling into rod-like structures at a portion of a

filament. Penetration of the stain occurred on some of them, giving the appearance of the rod-like structures seen in Fig. 1a (Fig. 1c). These results suggest that ether treatment causes swelling of the phage into a hollow cylindrical structure.

To investigate the chemical composition of Pf-1, phage was purified from infected culture fluid with the method of ammonium sulphate fractionation and equilibrium density gradient centrifugation of CsCl. Bacteria were removed from 2 l of fluid by centrifugation (8,500g for 30 min), 240 g of ammonium sulphate was added gradually with stirring to precipitate the phage.

The precipitate was collected by centrifugation (8,500g, 30 min) and dialysed against 0.01 M Tris-HCl buffer pH 7.4 overnight. The cell debris that remained in the phage

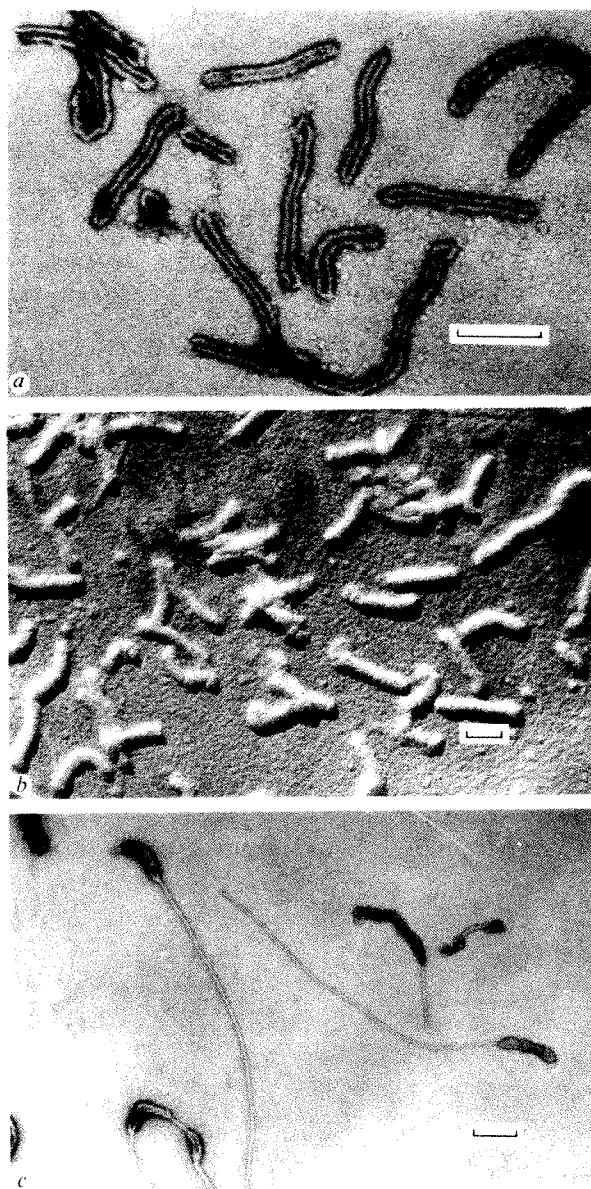


Fig. 1 Electron micrographs of Pf-1 treated with ether. *a*, Negatively stained Pf-1 treated with ether. The purified phage was mixed with an equal volume of ether for 10 min at room temperature. The aqueous phase was negatively stained with potassium phosphotungstate, pH 7.2, on the electron microscopic grid prepared as described by Fukami and Adachi⁸. Scale bar represents 100 nm. *b*, The same preparation of Pf-1 as in (*a*) was shadowed with tungstate at an angle of 30° using an Edwards E306 vacuum evaporator equipped with an electron beam device. *c*, Negatively stained Pf-1 treated with ether vapour. Purified phage was placed on specimen grids and exposed to ether vapour for 5 s by keeping the grids above the test tube containing ether.

suspension was removed by three cycles of differential centrifugation (20,000g, 10 min and 100,000g, 2 h). The gradient consisting of three steps (1.4, 1.3 and 1.2) and spun at 84,000g for 4 h. The phage band which appeared in the lower third of the gradient was collected, dialysed against 0.01 M Tris-HCl buffer overnight and further purified by CsCl equilibrium sedimentation (115,000g, 21 h). The phage band at 1.29 density was collected and dialysed against the buffer and this is referred to as the purified phage preparation. This phage preparation contained 1.6×10^{13} PFU per ml (plaque forming units) of phage which was 20% of the activity detected in the infected culture fluid. To demonstrate the purity of the phage preparation it was subjected to polyacrylamide gel electrophoresis containing sodium dodecyl sulphate (SDS) to separate the proteins following the method of Dunker and Rueckert⁴.

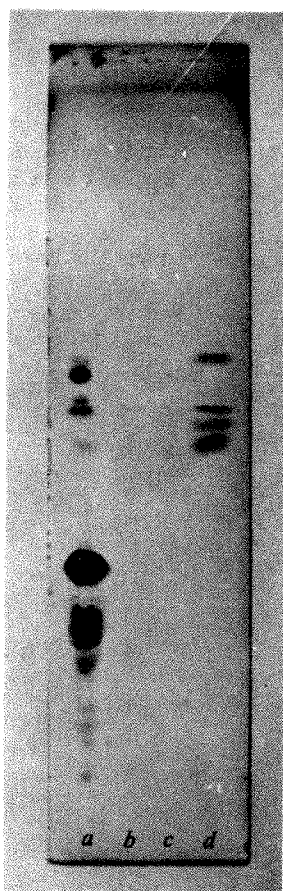


Fig. 2 Thin-layer chromatography of the lipids fraction extracted from Pf-1. Lipids were extracted from the purified phage suspension or bacterial cells by the method of Braunstein and Franklin⁵. Purified phage (1×10^{13} PFU) or bacterial cells (2×10^{10} cells) were suspended in 4.8 ml of 0.01 M Tris-HCl buffer, pH 7.4. Methanol-chloroform (2:1, v/v, 18 ml) was added to the suspension and intermittently shaken for 2 h at room temperature. The precipitated residue was centrifuged at 3,000g for 20 min and the supernatant retained. Extractions were repeated in the residue. Chloroform (6 ml) was added to the supernatant and mixed. Then 6 ml of water was added and also mixed. The emulsion was centrifuged at 3,000g for 30 min and the chloroform layer was collected, dried in room temperature and retained as the lipid fraction. Lipid extracts dissolved in the mixture of chloroform and methanol were applied 2 cm from one edge of a chromatographic plate. The plate was placed in a cylindrical jar containing 10 ml of chloroform-methanol-water (65:25:4, v/v) and allowed to stand until the front of the solvent reached 11 cm from the edge of the plate. The plate was air-dried and then transferred to the other jar containing 10 ml of the mixture of *n*-hexane-ethyl ether-acetic acid (90:10:1, v/v) and developed further until the solvent front reached 19 cm from the edge of the plate. The spots of lipid were visualised by staining with iodine or molibden blue. *a*, Lipid extracts from strain k of *Ps. aeruginosa*; *b*, lipid extracts from Pf-1; *c*, solvent alone; *d*, standard lipids, from top to bottom; triolein, diolein, cholesterol and oleic acid.

Only the two visible bands of protein which represented A protein and B protein respectively were stained. No other bands indicating the contamination of the proteins from bacteria were detected.

Lipid was extracted from the purified Pf-1 by the method of Braunstein and Franklin⁵ using 2.5 mg of phage (1×10^{13} PFU). Lipid fractions were analysed on a thin layer chromatography plate coated with silica gel 60 (Merck). Using the same plate the lipid fractions extracted from host bacteria (2×10^{10} cell) were analysed simultaneously. The plate was initially developed with a solvent mixture consisting of chloroform-methanol-water (65:25:4, v/v) and then with *n*-hexane-ethyl ether-acetic acid (90:10:1, v/v). The plate was stained with iodine or molibden blue; several spots were visualised on the specimens extracted from bacteria but none on the extracts from Pf-1. This implied that Pf-1, although it is sensitive to ether, does not contain significant amounts of lipid in its structure. Significant levels of lipids have been found in many other ether-sensitive viruses.

In lipid-containing viruses the morphological alterations induced by ether-treatment are thought to be caused by the extraction of the lipids which have a role in maintaining the morphological integrity of the virion. In Pf-1, the morphological alteration seems not to be the disintegration of the phage filament but its swelling into a hollow cylinder.

The structure of filamentous phages including Pf-1 has been extensively examined by X-ray diffraction and chemical analysis. A structural model has been presented by Marvin⁶.

The protein shell of the filamentous phage is constructed from coat protein molecules exhibiting largely an α -helix structure and extending in an axial direction with the phage filaments overlapping each other like fish scales to form a hollow cylindrical tube, in which the virus DNA is wrapped. In the phage protein shell, the coat protein molecules were held together by the hydrophobic bond created by the hydrophobic region of the coat protein molecules⁷. It is assumed that ether probably disrupts these bonds and induces deformation of the arrangement of the coat proteins on the protein shell and results in the swelling of phage filament. Our preliminary experiment to observe the DNA molecules in ether-treated phage specimens by the technique of protein monolayer method revealed the presence of many small circular DNA molecules similar in size and shape to those extracted by the phenol method from the purified Pf-1. This suggests that the DNA molecules packed in the protein shell might be released concomitantly with the swelling of the phage filament.

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Aflatoxin B₁-oxide generated by chemical or enzymic oxidation of aflatoxin B₁ causes guanine substitution in nucleic acids

AFLATOXIN B₁ (AFB₁) has the highest biological activity of the four naturally occurring aflatoxins produced by the

mould *Aspergillus flavus*¹. Feeding studies have shown it to be the most potent liver carcinogen known for the rat². Its ingestion by man is associated with the high primary liver cancer incidence in certain parts of Africa³. There is much evidence to suggest that AFB₁ requires metabolism to exert both its carcinogenic and mutagenic effects (reviewed in ref. 4). Structure-activity⁵, as well as chemical studies^{6,7} indicate that the major route for activation proceeds through mixed function oxidase attack yielding the 8,9-oxide (previously called the 2,3-oxide but renumbered according to IUPAC recommendations). Attempts to chemically synthesise this metabolite have so far been unsuccessful. We report here that peracid oxidation of AFB₁ generates AFB₁-8,9-oxide, and that this latter compound reacts readily with nucleic acids to give adducts identical to those obtained after liver mixed function oxidase (MFO) activation *in vitro* and *in vivo*.

Using a modification of a chemical procedure recommended for the epoxidation of acid sensitive olefins⁸, we are able to covalently bind AFB₁ to DNA, polyguanylic acid or deoxyguanosine. Enzymic binding to nucleic acid was achieved by incubating the carcinogen with rat or hamster liver post-mitochondrial supernatants and DNA together with the necessary cofactors.

Figure 1 shows the chromatographic profile on Sephadex LH-20 of deoxynucleosides obtained by enzymic hydrolysis of DNA after MFO activation of AFB₁ compared with that after chemical activation. Four peaks of radioactivity were obtained, in each case the major peak being product I. Freeze-drying of fractions from peaks I and II and injection onto a silica reversed-phase column and subsequent high pressure liquid chromatography showed each peak to be

homogeneous (data to be presented elsewhere).

Mild acid hydrolysis of AFB₁-reacted DNA, after MFO activation, to release purine bases and subsequent LH-20 chromatography of the hydrolysate, showed three peaks of radioactivity. Two of the peaks had similar retention times to the minor peaks (III and IV) obtained after enzymic hydrolysis. A similar chromatographic profile after mild acid hydrolysis was obtained for AFB₁-reacted DNA after chemical activation (data not shown).

Evidence for the structures of the various products of AFB₁ reaction with DNA was obtained by substituting deoxyguanosine or polyguanylic acid as nucleophiles, in place of DNA, in the chemical oxidation system. Chromatography of the deoxyguanosine reaction products on Sephadex LH-20 gave peaks of radioactivity coincident with peaks I, II and IV obtained in enzymic hydrolysis of DNA after either chemical or enzymic reaction with AFB₁ (Fig. 2). Incubation of the deoxyguanosine reaction products at pH 9.0 for 24 h and subsequent Sephadex LH-20 chromatography showed peaks II and IV to largely disappear and be replaced by peaks I and III. Strong acid hydrolysis (1 N HCl, for 60 min at 100 °C) of AFB₁-reacted polyguanylic acid from a chemical activation experiment and Sephadex LH-20 chromatography of the reaction products showed two major peaks of radioactivity (III and V) and a minor one (IV).

Thus, all the reaction products obtained after neutral enzymic hydrolysis of DNA, except for V, seem to be derived from guanine. Peak V is that of AFB₁-8,9-dihydrodiol, since it is excluded at the fifth void volume on Sephadex LH-20 chromatography⁹, and co-chromatographs with synthetically prepared diol. Acid hydrolysis of DNA

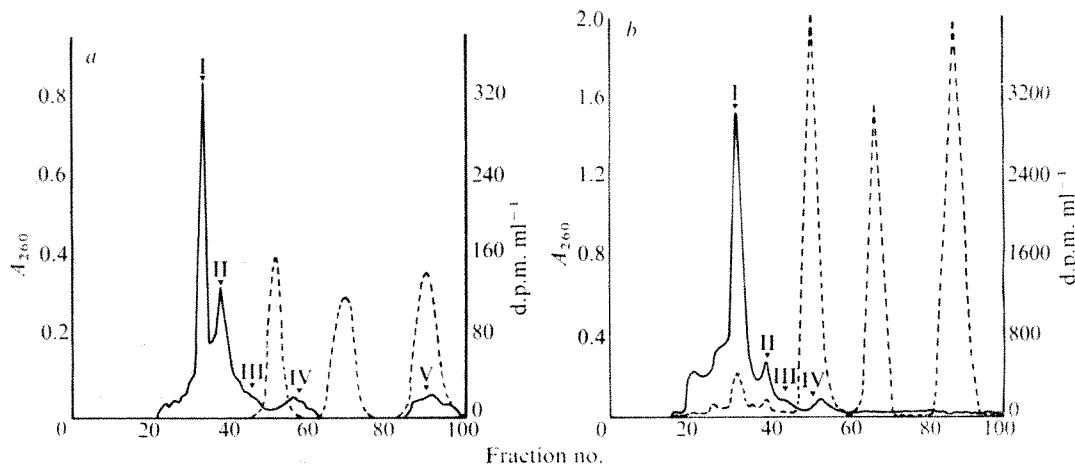


Fig. 1 Chromatographic profiles of neutral enzymic digests of DNA on Sephadex LH-20. —, ¹⁴C radioactivity; ---, A₂₆₀. *a*, Enzymic activation of AFB₁. Hamster or rat liver post-mitochondrial supernatant (1 ml of a 25% homogenate in 150 mM KCl, equivalent to 250 mg of fresh liver) was incubated in 100 mM sodium phosphate buffer pH 7.4 containing 1.5 μmol NADP⁺, 20 μmol glucose-6-phosphate, 25 μmol MgCl₂, 10 mg of calf thymus DNA and 200 μg ¹⁴C-AFB₁ dissolved in 0.2 ml dimethyl sulphoxide (specific radioactivity 0.52 mCi mmol⁻¹) in a total volume of 3 ml. After incubation at 37 °C for 60 min, the incubation mixture was extracted once with an equal volume of phenol-cresol, the nucleic acid precipitated from the aqueous layer with ethoxyethanol and redissolved in 5 ml sodium chloride-sodium acetate buffer. Contaminating RNA was removed by incubation with ribonuclease-A (50 μg ml⁻¹) and the DNA re-extracted with 0.5 volumes phenol-cresol. The nucleic acid was then precipitated, redissolved and chloroform extracted three times before centrifugation at 100,000g for 60 min to remove glycogen. 1.7 mg AFB₁-reacted DNA dissolved in 1 ml 0.3 M sodium chloride-0.25 M sodium acetate-0.015 M magnesium acetate was digested with 100 μg deoxyribonuclease I for 60 min at 37 °C. After the addition of 3 units bacterial alkaline phosphatase and 0.05 units snake venom phosphodiesterase the mixture was incubated for a further 18 h at pH 7.0 and 37 °C. *b*, Chemical oxidation of AFB₁. 10 mg of calf thymus DNA were dissolved in 8 ml 20 mM sodium phosphate buffer pH 7.4 and added to a solution containing ¹⁴C-AFB₁, dissolved in 8 ml dichloromethane (67.5 μg ml⁻¹ ¹⁴C-AFB₁, specific radioactivity 0.4 mCi mmol⁻¹ prepared as previously described¹¹). To this two-phase system was added 625 μg 3-chloroperbenzoic acid (4 molar excess) and the whole stirred for 25 h at room temperature in the dark in a stoppered flask. At the end of this time, the two phases were separated, and the aqueous phase, containing the nucleic acid, extracted five times with equal volumes of chloroform. The nucleic acid was then precipitated in three volumes of ethanol and redissolved in 20 mM Tris HCl pH 7.0. This ethanol precipitation step was repeated three times and the nucleic acid finally redissolved in 0.3 M sodium chloride/0.25 M sodium acetate. 4 mg of the AFB₁-reacted DNA dissolved in 1 ml sodium chloride-sodium acetate-magnesium acetate were digested using twice the enzyme concentrations in (*a*). Enzyme digests were chromatographed on Sephadex LH-20 columns (110 × 1.75 cm) eluted with water, 4-ml fractions collected and an aliquot counted for radioactivity. Absorbance was measured at 260 nm. The main ultraviolet absorbing peaks from left to right are deoxycytosine and thymidine combined, deoxyguanosine and deoxyadenosine. The results are from two similar columns run separately.

or poly G to which AFB₁ has been bound, leads to release of this derivative, showing that AFB₁-8,9-oxide is formed by either enzymic or chemical oxidation of AFB₁.

3-Chloroperbenzoic acid is a highly specific oxidising agent for the epoxidation of olefinic double bonds. Use of this reagent to epoxidise AFB₁ in the presence of DNA, results in a high percentage of nucleic acid bases being modified. No evidence was obtained for direct action of the peracid on DNA. In the conditions outlined here, one molecule of AFB₁ is bound per 100 molecules of base, thus, approximately one in every 20 guanine bases are modified. Increasing the amount of AFB₁ with a proportionate increase in the concentration of peracids leads to a higher level of binding. With liver MFO, lower amounts of binding were found but this was again dependent on the AFB₁ concentration.

Neutral enzymic hydrolysis of DNA leads to the release of deoxynucleoside adducts⁹, whereas mild acid hydrolysis results in the release of base adducts¹⁰. Peaks I and II seem therefore to be deoxyguanosine adducts of AFB₁-8,9-oxide whereas III and IV are guanine adducts of the epoxide. Mild acid hydrolysis (0.1 N HCl at 70 °C for 20 min) of peak I converts it to peak III (data not shown) suggesting

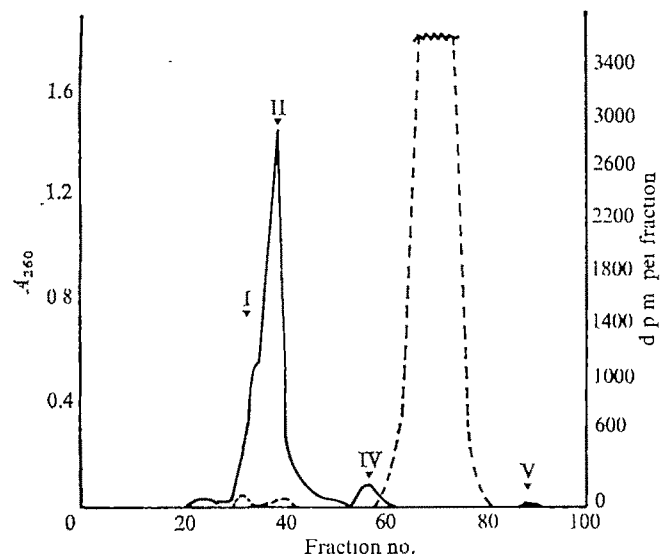


Fig. 2 Chromatographic profile of deoxyguanosine AFB₁-reacted adducts after chemical oxidation of AFB₁ at pH 7.4. —, ¹⁴C radioactivity; ---, A₂₈₀. Deoxyguanosine was reacted with AFB₁ as in Fig. 1b, with the exception of the ethanol precipitation step. Chromatography on Sephadex LH-20 was as in Fig. 1.

that peak III results from the loss of a sugar residue from peak I. Incubation of peak II at 37 °C, pH 9.0 converts it to peak I while peak IV is converted to III. Two explanations for this are that (1) alkali attacks the base-AFB₁ adduct to open the lactone ring, or (2) alkali attacks the adduct to open the imidazole ring of guanine. Explanation (1) is unlikely since peak I has the characteristic ultraviolet absorption maximum of AFB₁ between 360 and 370 nm and also incubation of AFB₁ at pH 9.0 does not result in lactone ring opening. Explanation (2) is substantiated by the absorption spectrum of peak I at pH 7.0 in the range 260–280 nm which is characteristic of a pyrimidine, suggesting destruction of the imidazole ring of the purine moiety.

Imidazole ring opening of guanine in mild alkaline conditions is characteristic of N-7 substituted guanines¹¹. Hydrolysis of AFB₁-reacted-DNA at neutrality for 30 min at 100 °C, results in the removal of the AFB₁-guanine adduct. Chromatography of the heat-hydrolysed supernatant after acid precipitation of the pyrimidine oligonucleotides at 0 °C on Sephadex LH-20 shows peaks III and

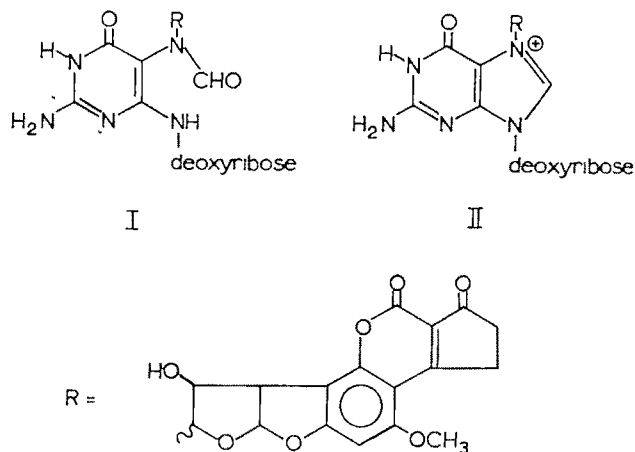


Fig. 3 Postulated structures of products I and II.

IV have been released (data not shown). Again this is a characteristic of guanine alkylated at the N-7 position¹².

Reaction at other positions in guanine is ruled out by (1) the relative ease of imidazole ring opening, (2) depurination at 100 °C in neutral conditions, (3) the ease of release of AFB₁-8,9-dihydrodiol from the nucleoside-AFB₁ adducts in mild acid conditions and (4) the base-AFB₁ adducts are stable at 100 °C in 1 N HCl. These findings lead us to assign the structures in Fig. 3 to the various products formed on reaction of AFB₁-8,9-oxide with DNA. Peaks I and II are the deoxyguanosine adducts whereas peaks III and IV are the guanine adducts. Data to be presented elsewhere shows, in agreement with other workers that products I to V are also formed on hydrolysis of liver DNA after ¹⁴C-aflatoxin B₁ administration to rats. Work is in progress to obtain sufficient of the products for chemical characterisation as well as identifying any possible minor reaction products.

The results reported here indicate that AFB₁-8,9-oxide is formed during peracid oxidation or MFO activation of AFB₁ and that this metabolite reacts with nucleic acid. Two major products of reaction are found, as has also been shown by Swenson *et al.*¹³, and these are both derived from guanine, almost certainly through N-7 substitution.

Covalent binding of AFB₁ with guanine at the N-7 position is likely to induce frame-shift mutations due to intercalation of the AFB₁ molecule together with base-substitution mutations as a result of misrepair. This reaction may therefore be important in the initiation of cancer as well as mutation induction in microorganisms by this potent mycotoxin.

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matters arising

Repression of synaptic efficacy in frog skeletal muscle

GRINNELL, Rheuben and Letinsky¹ show convincingly that when the transplanted frog sartorius muscle is innervated by two foreign nerves, control of the muscle fibres may be shared between the two, one nerve being effective at transmission the other eliciting only subthreshold junction potentials of low quantal content. They suggest this depends on a mutual competition between nerves similar to that invoked to explain selective reinnervation of urodele muscle^{2,3}.

I wonder whether this pattern of innervation is simply a property of the frog sartorius muscle. Luff and Proske⁴ have found using tension measurements, that there is very little overlap between motor units when they measure twitch tension but extensive overlap (50% or more) when tetani were used. Furthermore, motor units in which the twitch tension fluctuated from trial to trial required high rates of stimulation to achieve peak tetanic tension and had low twitch tetanus ratios. This suggests that normally many muscle fibres are supplied with junctions which have a low safety factor for neuromuscular transmission. Presumably those fibres are also supplied with a safe junction from another motoneurone. Since there are only about 12 motor axons supplying the sartorius it seems unlikely, although not impossible, that the reduced efficacy of some of the junctions depends on an embryological matching process such as that postulated to account for selective innervation of muscles after nerve regeneration in urodeles. Rather it may be because sartorius muscle fibres tend to maintain one neuromuscular junction at high efficacy and reduce the efficacy of others on the same fibre, regardless of the embryological origin of the synapses, an interesting developmental mechanism in its own right but not necessarily related to the formation of specific connections. One could answer the question by finding out whether in the preparation used by Grinnell *et al.* there were unequal connections made by different motoneurons within the two populations of foreign nerve fibres. The test would be to split one of the foreign nerves and see if there was evidence for competition between different populations of fibres in one nerve, as there is between

the populations of fibres of the two foreign nerves.

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¹ Grinnell, A. D., Rheuben, Mary B. & Letinsky, M. S. *Nature* 265, 368–370 (1976).

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³ Genat, B. R. & Mark, R. F. *Phil. Trans. R. Soc. B* 278, 335–346 (1977).

⁴ Luff, A. R. & Proske, U. J. *Physiol. Lond.* 258, 673–685 (1976).

GRINNELL *et al.* REPLY—Mark rightly asks the question: If competition between two different nerves reduces the effectiveness of many terminals, as we report, then should not the same phenomenon occur between axons of the same nerve? If so, might this not be a normal phenomenon whereby each fibre acquires (relatively) exclusive innervation by one axon? This was an obvious question to us as well, and we have done some experiments of the type he suggests.

Our results indicate that there is competition between axons of the same nerve, but much less than between axons of two different foreign nerves. When a single nerve (normal or reinnervated) is split into two or more segments, with one to three functional motor units in each, there is little if any twitch overlap (usually 5% or less), but up to 25% tetanus overlap. This implies the existence, in *Rana catesbeiana* as in *Litoria aurea*¹, of some subthreshold polyneuronal innervation, and leads us to agree with Mark that the competition we have demonstrated between pairs of foreign nerves may be a special case of similar competition between axons of a single nerve. In our experiments there is a sharp quantitative difference between competition in the two cases, however, and probably a qualitative difference. The tension overlap we find between axons in singly innervated muscles does not approach that reported by Luff and Proske⁴. The explanation for this difference may be one of species or technique. It is noteworthy, for example, that Luff and Proske's Ringer apparently contained only 1.08 mM Ca^{2+} compared with the 1.8 mM in our amphibian Ringer and that used by most other investigators. In four experiments in our laboratory, shifting a normal sartorius preparation from 1.8 mM Ca^{2+} to 1.08 mM Ca^{2+} Ringer caused no change in 50 per cent tetanus tension, but a reversible 53±

28% drop in twitch tension, suggesting that quantal content in a high proportion of junctions can be reduced below threshold by that reduction in Ca^{2+} concentration. This could also help account for Luff and Proske's low twitch-tetanus ratios. Although we find a few subthreshold junctions in singly reinnervated muscles, normally the quantal content is close to threshold, never as low as is commonly found in doubly innervated muscles (under 10). Moreover, the average twitch tension developed by simultaneous stimulation of both of two foreign nerves is conspicuously less (in percentage of fibres twitching) than that elicited by stimulation of the nerve in singly reinnervated muscles. This implies either fewer fibres are innervated, or that there is a greater number of subthreshold junctions where two different nerves are present rather than only one. Since we normally find that either nerve when tetanised can drive almost 100% of the muscle, most of the junctions formed by each must be subthreshold².

The competition we describe between the foreign somatic motor nerves is nonspecific; neither has an apparent innate advantage. Thus we do not propose this as a direct explanation for the reported instances of specific repression of one nerve by another. On the other hand, we do see some distinction between axons of two different nerves, compared with those of a single nerve. Moreover, the competition occurs in a muscle with focal rather than distributed endings, and hence is amenable to direct study of single 'repressed' endings. It is our hope that an understanding of this interaction may provide important insights into more specific forms of interaction.

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reviews

Jumping into black holes

Roman Znajek

The Iron Sun: Crossing the Universe Through Black Holes. By Adrian Berry. Pp. 176. (Jonathan Cape: London, 1977.) £3.95.

ADRIAN BERRY has written a book about making black holes and jumping into them. He claims that the latter is a way of achieving instantaneous interstellar transport. After reading half the book one eventually discovers that it is called *The Iron Sun* because Berry proposes to build the holes by using magnetic fields to sweep together bits of iron (and other junk) that float around between stars. As the holes are to be ten times as massive as the Sun, and are to be situated at the 'safe' distance of one light year, this is clearly going to be a colossal task. Berry, however, thinks that humanity will continue to increase its economic output at an average annual rate of 3 per cent, and will therefore be able to achieve this feat "within about 250 years' time". Opposition is to be expected from assorted politicians, conservationists and second-rate scientists, but Berry is nevertheless confident that the project will be successfully completed by a conspiracy of dedicated technocrats.

I should explain that a black hole is a region of space where gravity is so strong that nothing, not even light, can escape. Its formation is inevitable when a sufficiently compressed piece of matter gets too cold to support itself against its own gravitational field. A ten solar mass lump of iron will certainly start to collapse under its own weight. What Berry does not seem to realise is that it will also get very hot. It is possible that the outer regions will stop collapsing and then get blown away. In other words, Berry may have created a recipe for an artificial supernova. A supernova one light year away would be a thoroughly unpleasant object and the conservationists would be justified in opposing it. There is of course no point in building the hole much further away because it is precisely in order to travel across such distances that the hole is being built in the first place.

An optimist may say that this is a mere technical difficulty and that there might be some way of cooling the matter as it collapsed. So how is a black hole going to help us get to the

stars? According to Berry, whenever a black hole is formed, a white hole 'simultaneously' appears in another part of the Universe. (A white hole is the opposite of a black hole: instead of falling into it, matter tends to fall out.) The two holes are supposed to be connected by a short piece of space that is 'outside' normal space—a kind of cosmic Suez Canal. A properly navigated spaceship can disappear into the black hole and reappear an instant later coming out of the white hole, even though a beam of light would take many years over the same journey in ordinary space.

Berry admits that many problems must be solved before such a voyage is possible. He confesses at the end of the book that no one knows where the white hole is going to appear. The fact is that no one knows *why* the white hole is going to appear. The idea that black holes are linked to white holes is a speculation which very few relativists have taken seriously. Speculation is legitimate, but quoting it as established knowledge is not. Berry has performed a grave disservice to science by not passing on warnings like: "concepts outside our present knowledge of physics must be invoked" which regu-

larly appear in the references he relies on. He owns up to the instability of the short cuts through which his spaceships travel, but does not draw the appropriate conclusion that the short cuts simply don't exist.

There are very many errors in this book. Perhaps the worst is Berry's claim that absolute time exists in the theory of special relativity. He says that although "many people who should know better" deny it, Einstein himself believed in absolute time and the simultaneity of events. This is an important issue for Berry, because if absolute time did not exist his instantaneous form of transport would lead to logical paradoxes. Unfortunately, the page to which Berry refers in Einstein's *The Meaning of Relativity* appears to be about something else, and if Berry looks at the preceding page he will no doubt decide that Einstein too needs to know better . . .

Adrian Berry should have known better than to write this book. □

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Immunopathology

Textbook of Immunopathology. Second Edition. Vol. 1: Pp. xlviii+1-554. Vol. 2: Pp. xlviii+555-1,118. Edited by P. A. Miescher and H. J. Muller-Eberhard. (Grune and Stratton: New York and London, 1976.) \$49.50; £30.20 each volume.

THIS textbook of immunological mechanisms in disease and immunopathology has become a standard work in its field of medicine. It is indispensable to those who are involved in clinical immunology or in the study of animal disease models of immunologically mediated phenomena. A consortium of expertise from both sides of the Atlantic has contributed the 63 chapters contained in this two volume work. The quality of each is uniformly high. The format of the textbook has not been altered from the first edition; obviously the editors have not wished to tamper with success. Almost 400 new pages of text, however, have been

added, thereby expanding the volume by one-third. A final chapter, an appendage, on principles of methods in immunology seems out of place in this book, and is hardly an adequate source of information on methodology for investigators in the field. It is dissonant with the highly authoritative scholarship of the preceding 62 chapters.

Almost 75 years have elapsed since Metchnikoff's publication of lectures on immunopathology. He certainly would be gratified to see how his fundamental insights into immunopathology have blossomed into a complex, vital and growing field of medicine and medical research. I think that he too would have prized this textbook.

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Behaviourist history

Behaviourism and the Limits of Scientific Method. By Brian D. Mackenzie. Pp. 193 (Routledge and Kegan Paul: London and Henley, 1977.) £4.95.

IN *Behaviourism and the Limits of Scientific Method* Dr Mackenzie traces the history of behaviourism from Watson to Skinner and examines its philosophical assumptions. He notes that there have been several different kinds of behaviourist. Watson's insistence that in order to be a science, psychology should be concerned only with observable behaviour and not with the furniture of the mind was followed by a more general concern with scientific method, and many behaviourists went on to embrace the then fashionable reductionist doctrines of the logical positivists and operationists. The most extreme version of behaviourism is that still being promulgated in increasing isolation by B. F. Skinner, who abjures all theoretical concepts.

Dr Mackenzie makes some interesting points. He claims for example that the learning theorists of the thirties—Hull, Tolman and Guthrie—were led astray by their adherence to logical positivism. Because they believed that their theories were merely ways of systematising the data and that their explanatory concepts had no ontological status, they failed to make explicit many of the assumptions behind their theories: the mutual misunderstandings thus engendered led to many pointless wrangles. It is certainly true that, as they later came to realise, their theories lacked rigour, but the theoretical looseness surely arose less from their philosophical assumptions than from the fact that at that time no formal language existed in which to couch rigorous explanations of behaviour.

Dr Mackenzie fails to note the difference between postulating intervening variables and putting forward a mechanism to explain behaviour. It is ironic that psychologists should have been reluctant to reify their explanatory concepts. It is clear that real processes intervene between the stimulus and the response and these processes are instantiated in a corporeal body known as the brain. To understand the logic of the brain's connectivity, psychologists needed to evolve new concepts, but the reality of the brain cannot be doubted in the same way as it is possible to doubt the reality of quarks.

Although Dr Mackenzie has provided an interesting and original account of the philosophy and history of behav-

ourism, the book has one curious fault: he has kept his eye so firmly on his quarry that he seems unaware that there have always been psychologists of many different colours. He even refers to behaviourism as "the most important single influence in the continuing development of modern psychology". This is a startling assertion to make at a time when many psychologists explicitly take account of introspection, and when almost all are concerned with building process models. Behaviourism has in fact never had much effect on the study of perception, language or cognition,

even those studying memory have shaken off its influence over the past two decades. Moreover, at the time when Hull, Tolman and Guthrie were at their most influential, Lashley, Kohler, Wertheimer and Bartlett were putting forward ideas and theories of a very different kind. Dr Mackenzie clearly did not come to praise behaviourism: it is a pity he has not appreciated that he is too late to bury it.

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Photoconductive techniques

Photoconductivity and Related Phenomena. Edited by J. Mort and D. M. Pai. Pp. 502. (Elsevier, Amsterdam and New York, 1976.) Dfl. 155; \$61.95.

PHOTOCONDUCTIVITY is probably the most sensitive of all the electrical phenomena, to the defect properties of insulators and semi-insulators. Furthermore, the photoconductive technique can be studied under a wide range of experimental variables such as wavelength, intensity, temperature, applied electric field, and in steady-state and non-steady conditions. Consequently, photoconductive data are often very distinctive and rich in structure, providing a powerful probe for investigating the trap parameters and structure of solids, as well as an insight into physical processes involved such as absorption photogeneration recombination, transport, intramolecular relaxation, electro-photon interaction, and so on.

It is not surprising, therefore, that in recent years photoconductivity techniques have been increasingly utilised in the study of the disordered and amorphous state. This book describes the applications of photoconductive techniques to these materials, a collection of review articles by leading workers in the field.

The first nine chapters are devoted to basic concepts and experimental techniques. The book opens appropriately with a review of contact effects, often neglected and ignored (conveniently?), yet absolutely fundamental to a proper understanding of photoconductivity. Chapter 2 deals with experimental techniques, with particular attention on transient and time-varying Hall and photo-electromagnetic

techniques. Chapter 3 is concerned with the time-dependent photoconductivity, an area which I believe offers the greatest scope for future study, but which poses formidable problems. Chapters 4–9 deal with photoconductivity in a variety of materials: covalent semiconductors, molecular crystals, amorphous tetrahedrally bonded solids, amorphous chalcogenides, and polymeric photoconductors. The most comprehensive of these, and a very readable account, is the one on amorphous chalcogenide and non-polar liquids. There is surprisingly little duplication considering the closely-related nature of the work, and that the works are by different sets of authors, thus reflecting the wide-ranging versatility of photoconductive techniques.

The final two chapters are concerned with applications of photoconductivity. Chapter 10 deals with photoelectronic semiconductor devices. I would have liked to have seen more space devoted to this chapter, and more emphasis on devices using disordered photoconductors, because this is what the book is really all about; even if this were at the expense of those based on crystalline materials. The last chapter, a fitting close to the book, is concerned with electrophotography, and provides a good account of the basic principles of electrophotography, image formation and development, and requirements for photoreceptor materials.

On the whole, most of the articles are concerned with underlying physical processes stressed rather than the theory, and most are very readable. The book is prepared to a high standard, and should appeal to experts (and others) in the field, although I doubt very much if the price (\$62) will.

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Simplifying sociobiology

Sociobiology and Behaviour. By D. P. Barash. (Elsevier Scientific: New York, 1977) Hardback, \$9.95; paperback, \$4.95

Sociobiology and Behaviour is essentially a much abbreviated version of parts of E. O. Wilson's *Sociobiology. The New Synthesis* (Harvard University Press). After introducing the basic ideas of natural selection, Barash proceeds through the by now familiar topics: altruism, living in groups, mate selection, parental investment, and aggression. Although Barash covers the 'right' topics, is usually clear in what he says, and provides plenty of good examples, this is not a book I would recommend to first-year undergraduates, the intended audience.

Although I am all in favour of making scientific texts clear and informal in style, I find Barash's racy prose annoying. Phrases such as "tunnel of love" (sticklebacks nest), "shennanigans of a love-crazed prairie chicken" (lek behaviour), "slice of the sociobiological cake" (meaning uncertain) lack both elegance and precision. This, however, is a purely aesthetic reaction, and my real objections to the book arise from three aspects of its scientific content. First, Barash claims more for sociobiology than it deserves. He proudly proposes (for the first time, he says) the Central Theorem of Sociobiology—"animals should behave so as to maximise their inclusive fitness". It sounds to me rather like what Darwin said more than a hundred years ago. Second, the book sets a bad example to students by giving the wrong impression of what scientific prediction is all about. On p.259 we are told of the prediction of "sociobiologic theory" that "territories will be defended when it is adaptive for individuals to do so, i.e. when their personal inclusive fitness (*sic*) is maximised"! Almost as bad is one of the quoted 'tests' of this theory: wildebeest do not defend territories because it would not be adaptive for them to do so. It is a great pity that Barash gives readers the impression that "sociobiologic theory" (which I call natural selection) makes only tautologous or trivial and uninteresting predictions, because evolu-

tionary theory can be used to make highly quantitative and often counter-intuitive predictions just like any proper hypothesis.

My third criticism is that Barash, in his effort to make the book simple, often stops short of the point where he could convey some real understanding of the issues involved. Let me give a few examples. The discussion of costs and benefits of ritualised fighting (p.233) simply misses the point because it does not make clear the fact that the benefit to an individual derived from any particular strategy depends on what everyone else does. The payoffs are frequency dependent, so the problem is to find an evolutionary stable strategy. The same line of reasoning should have been applied to the review of mating systems (p.168 *et seq.*). In summarising the idea of sexual selection, Barash presents one hypothesis (the "handicap principle") without pointing out any of its known pitfalls, and fails to make a clear statement of the most widely accepted idea (Fisher's theory). The important work of Trivers and Hare on eusocial insects is incorrectly reported in a single sentence on p.84; the cost of sex is incorrectly explained on p.139; and although Barash asserts on p.119 that "gregariousness in shorebirds represents a unique and optimal compromise between conflicting demands of predation . . . and . . . foraging interference", the reference he cites has no information on whether or not spacing is optimal.

These points, and many other similar ones, are not too difficult to explain to first-year undergraduates (see *The Selfish Gene* by Richard Dawkins, Oxford University, 1976), and I feel that it is absolutely essential to get things exactly right if one is to convince students that evolutionary biology is more than a hand-waving exercise.

A final point about presentation. The text is well illustrated with good graphs, but inexplicably the photographs often have little to do with the text. A description of distraction displays in the Killdeer is accompanied by a photograph of two cranes, a pair of male Topis serves to illustrate territorial behaviour in the Wildebeest, and plate 3.3, a bizarre study of grey blobs, defies analysis.

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● The UK edition of *Neuronal Recognition*, by S. H. Barondes, has been published by Chapman and Hall (London), price £20. For review, see *Nature*, 267, 85, 1977.

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Hückel molecular orbital model

The HMO Model and its Application. Vol. 2: Problems with Solutions. Vol. 3: Tables of Hückel Molecular Orbitals. By E. Heilbronner and H. Bock. Translated by W. Martin and J. Anthony Rackstraw. Pp. viii+449 and viii+190. (Wiley-Interscience: London and New York; Verlag Chemie GmbH: Weinheim, 1976.) £15 and £9; \$33 and \$18.

THESE volumes complete Heilbronner and Bock's handbook on the Hückel molecular orbital model. Volume 1 set out the basic theory and techniques, and contained numerous problems; Volume 2, which is almost as long, contains only the solutions to those problems. Anyone who works through all the problems would acquire a very extensive knowledge of Hückel theory. I suspect, however, that this knowledge would be deficient in being rather mechanical, superficial and uncritical. It is typical, for example, that although the main text provides an adequate

general derivation of the pairing theorem, the associated problem requires the reader to prove that a crude approximate formula for Hückel energies also satisfies the theorem—a result of far less power and significance. Again, the authors provide a mechanical procedure for factorising a Hückel determinant with the help of the molecular symmetry. Not only is it mechanical; it is also unnecessarily laborious, since it involves a lengthy series of operations on the entire secular determinant. Not only that; it doesn't even work where there are degeneracies (try it for cyclopropenyl). Setting up the determinant directly in terms of symmetry-adapted functions is easier, more general, and more illuminating.

Hückel theory is well known to be a very poor approximation for many purposes. The authors point out some of its failings, but do not indicate how they may be overcome, even by providing a suitable reference. Examples are the failure to predict negative spin populations, and the false degeneracy of the second excited state of alternant hydrocarbons. These are both due to the failure of Hückel theory to take account of electron repulsion; however, in both cases a simple correction can

be made to improve the Hückel result.

The third volume contains tables of Hückel molecular orbital coefficients and derived quantities such as bond orders and atom-atom polarisabilities. It covers chains (up to 10 atoms), rings (up to 18 atoms), an assortment of alternant and non-alternant molecules with up to three rings, and the partial π systems obtained by removing one carbon atom from naphthalene, anthracene, phenanthrene, azulene and biphenylene. It is intended partly as an adjunct to the problems, many of which refer to the data in the tables; however, it is also more generally useful. On the other hand, when writing a Hückel molecular orbital programme is a straightforward undergraduate exercise it cannot be said that the need for such tables is very great.

In summary, the work cannot be regarded as a definitive treatise on Hückel theory: it omits too much. As an introduction it is reasonably thorough, although even here there are some omissions. A useful but not indispensable book. **A. J. Stone**

A. J. Stone is an Assistant Director of Research in Theoretical Chemistry at the University of Cambridge, UK.

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Plasma in gas

Plasma Phenomena in Gas Discharges. By R. N. Franklin. (Oxford Engineering Science Series.) Pp. ix+249. (Clarendon; Oxford: Oxford University: London, 1976.) £15.

LECTURE courses spawn books as readily as the proverbial salmon spawning eggs. Unfortunately graduate courses and the books they gestate too frequently limit themselves to the particular interests of the lecturer. This would seem to be the rationale behind this scholarly but narrow book by Raoul N. Franklin.

The book is largely concerned with analysing the positive column and illustrating the diversity of plasma phenomena which can occur therein. Though these small scale, low current, experiments are of great importance in the historical development of plasma physics, they nevertheless represent a very small area in what is now a much wider experimental field.

In the first half of the book detailed solutions of radial equilibrium, of space-charge effects at walls and electrodes and of the influence of a magnetic field in a positive column are presented. The second half of the book rests a little uneasily on the first part and aims to describe the variety

of plasma phenomena that can be observed in gas discharges. It is largely, however, a catalogue of linear plasma waves, including electron plasma waves, ion waves, ionization waves, magnetosonic and 'whistler' waves and drift waves. As a description of wave phenomena in plasmas, it is a useful and reasonably comprehensive compendium, though at this level it must be compared with many excellent books on waves in plasmas. I would have been happier with more physical insight, discussion and interpretation of the equations used and results obtained, as opposed to the *ad hoc* writing down of equations, linearisation and solution of the resultant dispersion relations. As a wide catalogue and a clear analysis of waves in plasma it is useful. A helpful feature is the inclusion of comparisons with experiment whenever possible and a wide range of references to experimental work.

Though the editing can be faulted and the production is dull, the book is scholarly and precise. It is certainly a useful text for those working directly in gas discharges but of less value to the wider student of plasma physics.

David Potter

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